Studies on *Phaseolus vulgaris* phytohemagglutinin

Structural requirements for simple sugars to inhibit the agglutination of human group A erythrocytes

Gilles Dupuis and Benoît Leclair

Département de Biochimie, Université de Sherbrooke, Sherbrooke, Québec, J1H 5N4 Canada

Received 7 May 1982

Phytohemagglutinin specificity *Erythroagglutination* *Haptenic sugars*

1. INTRODUCTION

Lectins are proteins which can act as cell agglutinins and, in some cases, can induce lymphocyte transformation [1–3]. These properties are related to their ability to act as sugar-recognizing proteins. A classification of lectins, based on their sugar specificity, has been proposed [4]. Phytohemagglutinin (PHA), the lectin from *Phaseolus vulgaris* (red kidney beans), possesses a large sugar binding site which enables recognition of complex oligosaccharidic structures at the cell surface [5–7]. There are conflicting reports concerning the ability of PHA to specifically recognize monosaccharides [5–14]. Furthermore, it has been claimed that the lectin does not show specificity towards agglutination of human erythrocytes [4,12].

Here, we have investigated the specificity of PHA towards agglutination of the major human erythrocyte blood groups using the technique in [15]. Our results show that PHA agglutinates preferentially human erythrocytes of group A. A series of monosaccharides have been used to assess the structural requirements for monosaccharides to inhibit the agglutination reaction. Results show that the lectin can distinguish between opposite configuration or substitution at positions 2 and 4 of the pyranose ring.

Abbreviations: PHA phytohemagglutinin; PBSA, phosphate-buffered saline–bovine serum albumin

2. MATERIALS AND METHODS

Phytohemagglutinin prepared according to [16], was purified on a Sepharose–thyroglobulin column as in [17]. The lectin appeared to be homogeneous by polyacrylamide gel electrophoretic analysis at pH 9.3 and 4.3. Mono-, di-, tri- and tetrasaccharides were obtained from Sigma (St Louis MO) or from PL-Biochemicals (Milwaukee WI). 1-Amino-1-deoxy-D-galactopyranose was prepared as in [18].

Blood obtained by venipuncture was centrifuged (10 min/600 × *g*), plasma and buffy coat were removed and the erythrocyte suspension was diluted (1:10) with Dulbecco's phosphate-buffered saline containing 0.5% (w/v) bovine serum albumin (PBSA) and centrifuged. An additional wash with PBSA was performed and the red blood cells were diluted to 2×10^6/ml.

Agglutination assays were performed according to [15]. Various amounts of PHA (1 mg/ml) in phosphate-buffered saline were deposited, in duplicate, in the wells of a 'tissue cluster' plastic box (Costar, Cambridge MA) and the volume was completed to 0.1 ml with PBSA. Erythrocytes (2×10^5 cells) in PBSA (0.1 ml) where then added. The box was gently agitated (wrist-action shaker) overnight, at 4°C. The cells were carefully aspirated with a short (14.5 cm) Pasteur pipette and deposited into a spectrophotometric cuvette. Volume was completed to 1 ml by addition of PBSA (0.8 ml). Readings were performed at 550 nm. An interval of ~3 s elapsed between dilution and absorbance readings. For inhibition of agglutination, PHA (8–10 μg) was added to individual wells of the tissue

Published by Elsevier Biomedical Press
00145793/82/0000–0000/$2.75

culture dish, followed by addition of a 0.3–1.0 M solution of saccharide in PBSA. The volume was completed to 0.1 ml and the mixture was left, under agitation, for 30 min at 4°C. Red blood cells (group A) were then added (2×10^5 cells) and incubation and readings were performed as above.

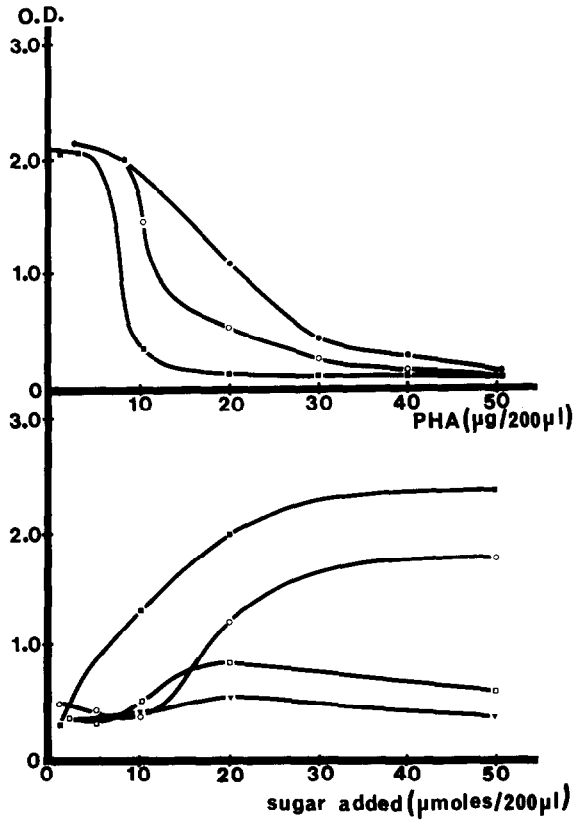

Fig.1. Upper: Turbidimetric measurements of human erythrocyte suspensions (2×10^5 cells/ml) as a function of increasing amounts of PHA, in 0.2 ml total vol. The mixture was incubated overnight at 4°C in a multi-well plastic dish, each well was diluted to 1.0 ml before readings were done. The graphs represent the effect of PHA on the agglutination of human cells of group A (▪), group O (○) and group B (•). Lower: Various amounts of test saccharides were added to a solution of PHA (8–10 µg). The mixture was incubated for 30 min at 4°C and human erythrocytes (type A) (2×10^5 cells) were added: final vol. 0.21 ml. Conditions of incubation and readings were done as above. The graph represents the effect of 2-acetamido-2-deoxy-D-galactose (▪), D-galactose (○), 2-acetamido-2-deoxy- D-glucose (□) and 2-amino-2-deoxy-D-galactose (▾) on PHA-induced erythroagglutination. Ordinate represents readings in A_{550} units.

3. RESULTS

The results of human erythrocyte agglutination are presented in the upper part of fig.1. PHA agglutinated erythrocytes from the major human blood groups but was more efficient towards cells of group A. Concentrations of lectin necessary to bring 50% agglutination (absorbance units) of the cells were 8 µg (group A), 12 µg (group 0) and 22 µg (group B), whereas complete agglutination was observed with 15 µg (group A), 40 µg (group 0) and 40–50 µg (group B) of lectin. The effect of various saccharides on the agglutination reaction is shown in the lower part of fig.1. Results show that, in addition to 2-acetamido-2-deoxy-D-galactose, D-galactose is a good inhibitor of the agglutination

Table 1

Inhibition of PHA-induced agglutination of human erythrocytes (group A) by various sugars

Sugar added	Quantity of sugar to produce a 50% inhibition of agglutination (µmol)
2-Acetamido-2-deoxy-D-galactose	8–10
D-Galactose	22
1-Amino-1-deoxy-β-D-galactopyranose	30
D-Fucose	30
D-Glucose	> 50
D-Fructose	> 50
D-Mannose	> 50
2-Amino-2-deoxy-D-galactose	> 50
2-Amino-2-deoxy-D-glucose	> 50
2-Acetamido-2-deoxy-D-glucose	> 50
1-*O*-Methyl-β-D-galactopyranose	> 50
Lactose	> 75
Lactulose	> 75
Raffinose	> 75
Stachyose	> 75

The test sugar was added to a solution of PHA (8–10 µg) in PBSA and incubations were performed for 30 min at 4°C. Human (group A) erythrocytes (2×10^5 cells) were added, the volume was completed to 0.2 ml and incubation was continued overnight at 4°C. The mixture was diluted to 1.0 ml and turbidimetric readings at 550 nm were performed

reaction. In this typical experiment, 2-acetamido-2-deoxy-D-glucopyranose and 2-amino-2-deoxy-galactopyranose are inactive. The effects of a series of saccharides on the agglutination reaction is shown in table 1 where the no. μmol sugar necessary to produce a 50% inhibition (absorbance units) of agglutination of human group A erythrocytes are tabulated. There was no inhibition of agglutination by monosaccharides such as D-glucose, D-fructose, D-mannose, 1-*O*-methyl-β-D-galactopyranose, 2-amino-2-deoxy-D-glucose, 2-amino-2-deoxy-D-galactose and 2-acetamido-2-deoxy-D-glucose. Di-, tri- and tetrasaccharides were also tested (table 1). Lactose (*O*-β-D- galactopyranosyl- (1→4)-D-glucopyranose), lactulose (*O*-β-D-galacto-pyranosyl-(1→4) -D-fructofuranose), raffinose (*O*-α-D-glucopyranosyl-(1→5)-D-fructofuranose) and stachyose (*O*-α-D-galactopyranosyl-(1→6)-α-D-galactopyranosyl-(1→6) -α-D-glucopyranosyl-(1→5)-β-D-fructofuranose) were not inhibitors. It is interesing to compare the inhibitory effect of 1-amino-1-deoxy-β-D-galactopyranose and 2-amino-2-deoxy-D-galactose. Whereas the former was inhibitor of agglutination, the latter had no effect.

4. DISCUSSION

The technique of inhibition of lectin-induced agglutination of erythrocytes, has been used for topological examination of the sugar binding site and specificity of lectins (reviewed in [4]). Kornfeld and Kornfeld [5,6] have isolated a glycopeptide from human erythrocytes and shown that it inhibits PHA-induced agglutination of human erythrocytes. They proposed the structure *O*-β-D-galactopyranosyl-(1→4)-2-acetamido-2-deoxy-D-glycopyranosyl-(1→2)-D-manno-pyranose for this saccharide. Toyoshima et al. [7] used glycopeptides derived from porcine thyroglobulin to study inhibition of PHA-induced stimulation of human lymphocytes and agglutination of human erythrocytes. They concluded that the determinant sugar had the same structure as proposed in [5,6]. Kaif and Osawa [8] have synthesized the trisaccharide mentioned above and shown that it inhibits PHA agglutination of human erythrocytes. These authors have also studied the effect of chain shortening. Whereas, the disaccharide *O*-β-D-galactopyranosyl-(1→4)-2-acetamido-2-deoxy-D-glucopyranose was an effective inhibitor, *O*-β-D-2-acetamido-2-deoxy-D-glucopyranose-(1→2)-D-mannopyranose was ~3-times less active than the trisaccharide.

The above results would suggest that the sugar binding site of PHA can accommodate a large oligosaccharidic structure bearing, at least, the D-gal-D-glcNAc structure. However, there are contradictory reports in the literature with respect to the ability of PHA to recognize monosaccharides. Kornfeld and Kornfeld [5,6] and Toyoshima et al. [7] have presented evidence that simple sugars were weakly recognized by PHA. In contrast, Borberg et al. [9,11], Pogo [10] and Kay [14] have shown that 2-acetamido-2-deoxy-D-galactose interferes with the biological activities of PHA.

Here, we have used a PHA preparation which migrates as a single band on electrophoretic gels. Agglutination assays (or inhibition) was performed overnight at 4°C. This approach resulted in reproducible spectrophotometric determinations.

Our results show that PHA selectively agglutinates erythrocytes of the major human blood groups. Group A red blood cells are best agglutinated by the lectin, group O is less strongly agglutinated and, group B, the least of the 3. Our data confirm the reports that 2-acetamido-2-deoxy-D-galactose can serve as an effective inhibitor of PHA-induced agglutination of human erythrocytes but extend the studies to a determination of the requirements at positions 1,2,4 and 6 of the pyranose ring. We observed that 8–10 μmol 2-acetamido-2-deoxy-D-galactose was sufficient to inhibit the agglutination reaction by 50% in terms of absorbance units. When position 2 of the galactopyrasone ring bears a hydroxyl function, the sugar (D-galactose) is ~3-times less active than its acetamido analog. Replacement of the hydroxyl function at position 6 by a hydrogen atom diminishes but does not abolish haptenic activity. The sugare (D-fucose) is 3–4-times less effective than 2-acetamido-2-deoxy-D-galactose. Interestingly, when position 2 is substituted by a positively charged amino group (2-amino-2-deoxy-D-galactose), haptenic activity is lost. This is not the case for position 1 where the hydroxyl group of the hemiacetal function can be replaced by an amino group. Configuration at position 4 appears to be of great importance. Epimerisation at this position yields the non haptenic D-glucose and 2-acetamido-2-deoxy-D-glucose. D-mannose, which is epimeric with D-galactose at positions 2 and 4, was not active. Similarly, D-fruc-

tose was inactive. Di, tri- and tetrasaccharides bearing the sequence D-galactose–D-glucose, D-galactose–D-fructose, D-glucose–D-fructose, or D-galactose–D-galactose–D-glucose–D-fructose were not active.

It would appear that PHA possesses, at its sugar binding site, at least one locus which recognizes and discriminates specifically between the nature and configuration of substituents at positions 2 and 4 of the pyranose ring. Our data do not allow an assessment of the conformational requirements for position 3. The finding that 1-*O*-methyl-β-D-galactopyranose was not inhibitor was unexpected in view of the fact that in the case of other lectins, the 1-*O*-methyl derivatives of sugars can usually act as haptens [4]. The lectin BS II from *Bandeiraea simplicifolia* can discriminate between the two anomeric *O*-methyl-D-glucopyranosides [19] but it remains to be seen if PHA can show the same specificity towards the *O*-methyl derivatives of D-galactose.

Our findings that PHA can selectively agglutinate erythrocytes of the major human blood groups is in agreement with the observed specificity of the lectin towards monosaccharides. The terminal sugars of the carbohydrates moieties of the major human blood groups are: *O*-α-2-acetamido- 2-deoxy-D-galactopyranosyl-(1→3)-β-D-galactopyranosyl (group A); *O*-α-D-galactopyranosyl-(1→3)-β-D-galactopyranosyl (group B); and *O*-β-D-galactopyranosyl-(1→3)-β-2-acetamido-2-deoxy-D-glucopyranosyl (group O(H)) [4,20]. These structural relationships can, in part, explain the specificity of PHA towards selective agglutination of human erythrocytes as reported in this study. Additional factors such as the size of the lectin sugar binding site and the number of sugar residues which an be accommodated, remain to be investigated.

Work is in progress in our laboratory to label the sugar binding of PHA with suitable derivatives of D-galactose.

ACKNOWLEDGEMENTS

This work was supported by the Medical Research Council of Canada (grant MT-6343) and by a grant from the 'Programme Institutionnel à la Recherche Libre' from the 'Université de Sherbrooke'.

REFERENCES

[1] Sharon, N. and Lis, H. (1972) Science 177, 949–959.
[2] Lis, H. and Sharon, N. (1973) Annu. Rev. Biochem. 42, 541–574.
[3] Brown, J.C. and Hunt, R.C. (1978) Int. Rev. Cytol. 52, 277–349.
[4] Goldstein, I.J. and Hayes, C.E. (1978) Adv. Carbohydrate Chem. Biochem. 35, 127–340.
[5] Kornfeld, S. and Kornfeld, R. (1969) Proc. Natl. Acad. Sci. USA 63, 1439–1446.
[6] Kornfeld, R. and Kornfeld, S. (1970) J. Biol. Chem. 245, 2536–2545.
[7] Toyoshima, S., Fukuda, M. and Osawa, T. (1972) Biochemistry 11, 4000–4005.
[8] Kaifu, R. and Osawa, T. (1976) Carbohydrate Res. 52, 179–185.
[9] Borberg, H., Woodruff, J., Hirshchorn, R., Gesner, B., Miescher, P. and Silber, R. (1966) Science 154, 1019–1020.
[10] Pogo, B.G.T. (1969) J. Cell. Biol. 40, 571–575.
[11] Borberg, H., Yesner, I., Gesner, B. and Silber, R. (1968) Blood 31, 747–757.
[12] Serafini-Cessi, F. (1980) FEBS Lett. 114, 299–301.
[13] Serafini-Cessi, F., Franceschi, C. and Sperti, S. (1979) Biochem. J. 183, 381–388.
[14] Kay, J.E. (1971) in: Proc. IV Annu. Leucocyte Culture Conf. (McIntyre, O.R. ed), pp. 21–35, Appleton-Century-Crofts, New York.
[15] Liener, I.E. (1955) Arch. Biochem. Biophys. 54, 223–231.
[16] Rigas, D.A. and Osgood, E.E. (1955) J. Biol. Chem. 212, 607–617.
[17] Felsted, R.L., Leavitt, R.D. and Bachur, N.R. (1975) Biochim. Biophys. Acta 405, 72–81.
[18] Barker, R., Chiang, C.-K., Trayer, I.P. and Hill, R.L. (1974) Methods Enzymol. 34, 317–328.
[19] Esibu, S., Iyer, P.N.S. and Goldstein, I.J. (1978) Carbohydrate Res. 61, 129–138.
[20] Schachter, H. and Roseman, S. (1981) in: The Biochemistry of Glycoproteins and Proteoglycans (Lennarz, W.J. ed) pp. 85–160, Plenum, New York.