RELATIONSHIP BETWEEN RED BLOOD CELL AGGLUTINATION AND POLYSACCHARIDE PRECIPITATION BY PHYTOHEMAGGLUTININ OF *PISUM SATIVUM* L.*

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1. Introduction

In addition to hemagglutinating activity, some non-specific phytohemagglutinins precipitate various polysaccharides. The similarity of both these processes to antigen-antibody interaction was suggested [1]. Interaction of phytohemagglutinins with various polysaccharides was extensively studied, especially in the case of concanavalin A, the phytohemagglutinin of Jack Bean [2–4]. A previous communication described a similar property for non-specific phytohemagglutinins of *Pisum sativum* and *Lens esculenta* [5]. Certain sugars affected both hemagglutination [6] and polysaccharide precipitation [7].

However, as far we know, the action of sugars on these systems was not correlated. In the present paper sugars were compared in regard to their capacity to inhibit hemagglutination and polysaccharide precipitation.

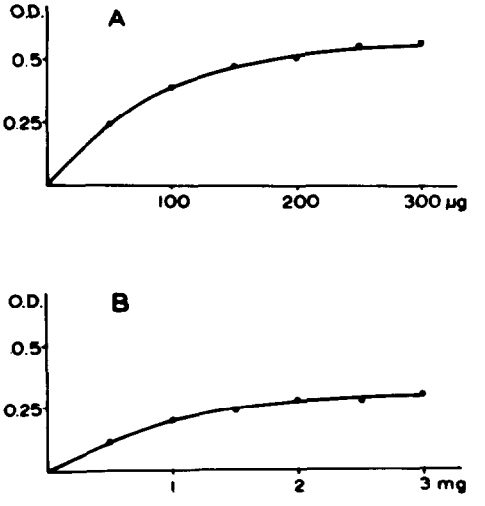

Fig. 1. Turbidimetric estimation of the precipitate formed by increasing concentration of polysaccharides. (A) precipitation of yeast mannan. 50–300 µg of the polysaccharide and 2 mg of phytohemagglutinin in 2 ml final volume of 0.05 M phosphate buffer pH 7.0; (B) precipitation of muscle glycogen. 0.5–3 mg of the polysaccharide and 4 mg of phytohemagglutinin in 2 ml final volume of 0.05 M phosphate buffer pH 7.0.

2. Materials and methods

Freeze-dried preparations of phytohemagglutinin from *Pisum sativum* was prepared by the procedure reported [5]. Yeast mannan from *Saccharomyces cerevisiae* of approximately 24,000 MW was a gift of Dr. Šikl, Chemical Institute, Slovak Academy of Sciences, Bratislava, Czechoslovakia. Muscle glycogen was purchased from L.Light and Co., Colnbrook, England, monosaccharides from Calbiochem., Los Angeles, California.

Phytohemagglutinin in 0.05 M phosphate buffer, pH 7.0 in the presence or absence of the monosaccharide inhibitor, was mixed with a solution of the polysaccharide in the same buffer and the volume made up to 2 ml. The development of turbidity was examined

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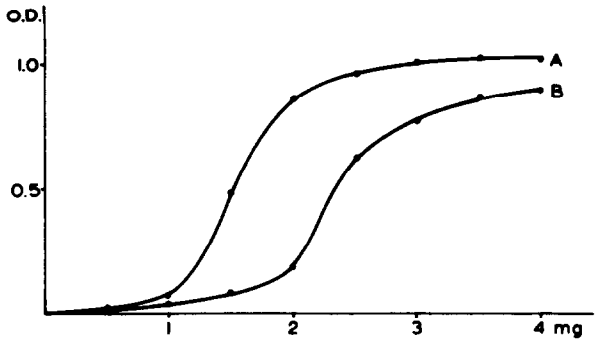

Fig. 2. Turbidimetric estimation of the precipitate formed by increasing concentration of phytohemagglutinin. 0.5–4 mg of phytohemagglutinin and 100 μg of mannan (A) or 2 mg of glycogen (B) in 2 ml final volume of 0.05 M phosphate buffer pH 7.0.

spectrophotometrically at various time intervals between 10–100 min with SF-4 spectrophotometer at 420 nm. The appropriate blank readings were obtained in the case of the mannan precipitation after addition of 0.1 ml of 2% D-mannose solution. The amount of D-mannose was sufficient to dissolve completely the mannan-phytohemagglutinin complex. Blank readings for glycogen precipitation were obtained by the addition of D-mannose solution before the formation of the glycogen-phytohemagglutinin precipitate. Subsequent addition of D-mannose did not dissolve the pre-formed precipitate.

The inhibition effect of sugars on hemagglutination was studied by the method reported by Tobiška [6] (see legend to fig. 5). Group O red blood cells washed three times were used.

3. Results and discussion

The reaction of phytohemagglutinin with different amounts of mannan and glycogen is depicted in fig. 1. Increasing turbidity with increasing phytohemagglutinin concentration and a constant amount of polysaccharides is shown in fig. 2. The rate of development of turbidity was essentially the same as described for concanavalin A by Poretz and Goldstein [2]. The most convenient time for reading turbidity was 40 min. On the basis of results presented in figs. 1 and 2, the optimal concentrations of the components for inhibition studies were chosen.

The ability of sugars to inhibit the mannan precipitation was in the order: D-mannose > D-glucose > D-fructose > D-galactose > L-arabinose (fig. 3). The same order was observed for glycogen precipitation (fig. 4).

The ability of sugars to inhibit red blood cell agglutination decreases in the same order, as the ability to inhibit precipitation of mannan or glycogen. The shape of curves characterizing hemagglutination inhibition is very similar to that obtained for the inhibition of polysaccharide precipitation (fig. 5). The sensitivity of phytohemagglutinin to inhibition by sugars in either the red blood cell agglutination or polysaccharide precipitation system, is apparent from the slopes of inhibition curves. The degree of inhibition decreases in the order: red blood cell agglutination, glycogen precipitation and mannan precipitation.

These results show that sugars inhibit the phytohemagglutinin-polysaccharide interaction in the same way as they do hemagglutination and this suggests

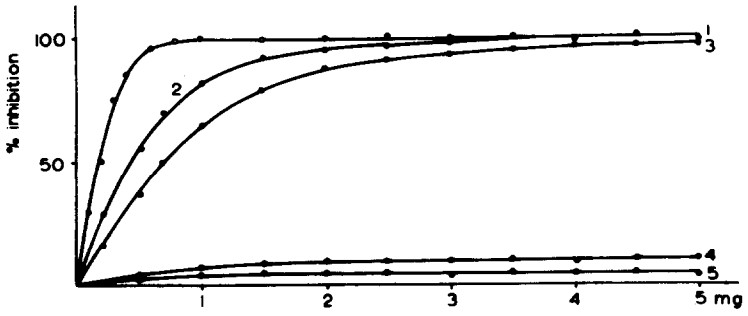

Fig. 3. Inhibition effect of sugars on yeast mannan precipitation. 100 μg of mannan, 1.5 mg of phytohemagglutinin and 0–5 mg of a sugar in 2 ml of final volume of 0.05 M phosphate buffer pH 7.0, 1) D-mannose, 2) D-glucose, 3) D-fructose, 4) D-galactose, 5) L-arabinose.

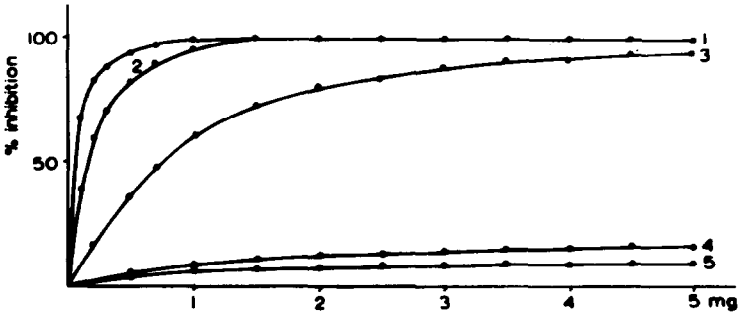

Fig. 4. Inhibition effect of sugars on muscle glycogen precipitation. 2 mg of glycogen, 3.5 mg of phytohemagglutinin and 0–5 mg of a sugar in 2 ml of final volume of 0.05 M phosphate buffer pH 7.0. 1) D-mannose, 2) D-glucose, 3) D-fructose, 4) D-galactose, 5) L-arabinose.

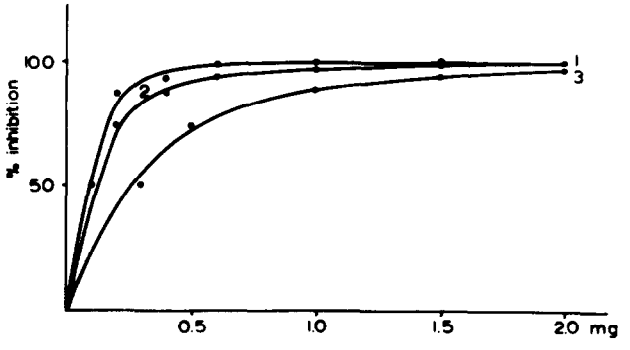

Fig. 5. Inhibition of red blood cell agglutination by sugars. 0.1 ml of double diluted 1% solution of phytohemagglutinin in normal saline and 0.1 ml of solution of appropriate concentration of sugar were mixed and after 15 min at room temperature, 0.2 ml of 2% normal saline suspension of red blood cells was added. After 15 min and centrifugation, the agglutination was observed macroscopically. 1) D-mannose, 2) D-glucose, 3) D-fructose. D-galactose and L-arabinose do not inhibit.

that the red cell receptor sites for phytohemagglutinin may be represented in the polysaccharides tested.

References

[1] J.B.Summer and S.F.Howell, J. Bacteriol. 32 (1936) 227.
[2] R.D.Poretz and I.J.Goldstein, Immunology 14 (1968) 165.
[3] B.B.L.Agrawal and I.J.Goldstein, Can. J. Biochem. 46 (1968) 1147.
[4] L.L.So and I.J.Goldstein, J. Biol. Chem. 242 (1967) 1617.
[5] G.Entlicher, M.Tichá, J.V.Kostíř and J.Kocourek, Experientia 25 (1969) 17.
[6] J.Tobiška, Die Phythämagglutinine, Hämatologie und Bluttransfusionswesen, Vol. 3 (Akademie Verlag, Berlin, 1964) p. 175.
[7] I.J.Goldstein, C.E.Hollerman and E.E.Smith, Biochemistry 4 (1965) 876.