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AGGLUTINATION ASSAY OF LECTINS IN PRESENCE OF SPECIFIC SUGARS BY USING BORATE

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SUMMARY

Incorporation of borate anion allows the agglutination activity of a lectin to be observed even in the presence of the specific sugar. This simple, convenient procedure worked successfully with five different lectins with different sugar specificities.

INTRODUCTION

The purifications of lectins generally involve their elution from an affinity matrix using a specific sugar (Pusztai, 1991). The removal of the sugar is necessary before the lectin is assayed by blood cell agglutination. We discuss below a simple modification of the agglutination assay in which incorporation of borate makes it possible to assay lectins without removal of the sugars.

MATERIALS AND METHODS

Concanavalin A, α-methyl-D-mannoside and N-acetylglucosamine were purchased from Sigma Chemical Co.(St.Louis, USA). D-Galactose was purchased from E.Merck (Bombay, India). D-Glucose and boric acid were purchased from Sarabhai M. Chemical (India). Glutaraldehyde (25%, w/v) was a product of Riedel-Dehaeneg Sellza, FRG. Wheat germ agglutinin, peanut agglutinin, soybean agglutinin and jack fruit seed agglutinin were isolated in our laboratory by using the procedures described in the literature (Vretblad, 1976; Pujol and Cesari, 1986; Lis and Sharon, 1972; Kumar et al., 1982).

Agglutination assay: Erythrocytes from rabbit blood were trypsinized and washed with saline as described by Lis and Sharon (1972). The trypsinized cells were treated with glutaraldehyde (0.1%, v/v) for 15 minutes and then washed with saline to remove excess glutaraldehyde (Turner and Liener, 1975). Finally, the cells were suspended in phosphate-buffered saline (6 mM potassium sodium phosphate buffer, pH 7.4, in 0.9% NaCl) and stored in refrigerator.

To assay a lectin in the presence of sugar, 50μl of lectin was first incubated with 50μl of specific sugar for 15 minutes at 37^{o}C and then 50μl of 0.05 M boric acid was added. After 15 minutes incubation at 37^{o}C, the lectin activity was checked using glutaraldehyde treated rabbit erythrocytes. However, as wheat germ agglutinin and peanut agglutinin were eluted by specific sugars from their affinity matrices, the boric acid was added directly in the effluent fractions and after 15 minutes incubation at 37^{o}C their hemagglutinating activities were tested.

Apparently, the procedure does not work with blood cells of all the animals as it did not work with human blood cells.

RESULTS AND DISCUSSION

The affinity of borate for carbohydrates is well known. The results summarized in Table 1 show that addition of adequate borate breaks up lectin-sugar complexes and lectin is free to agglutinate blood cells. Thus addition of borate allows the agglutination assay for lectins to be carried out without removal of the specific sugars. The procedure has worked for five lectins which were tried and should prove to be a general one. These five lectins were purposely chosen so as to try the procedure with lectins having three different and most commonly observed sugar specificities viz. towards glucose, galactose and N-acetyl glucosamine.

This modified agglutination assay was found to be simple, convenient and reproducible in our laboratory during isolation and characterization of lectins. The method was particularly useful in screening the sugar containing

TABLE 1

AGGLUTINATION OF LECTINS IN THE PRESENCE OF SUGAR AND/OR BORATE

For peanut and wheat germ agglutinins, the sugar concentrations were taken as 0.05 and 0.15 M as these lectins were eluted from affinity matrices using these concentrations. For jack fruit seed and soybean agglutinins those sugar concentrations were taken which are generally used in their elution during affinity chromatography (Lis and Sharon, 1972; Kumar et al., 1982). For Con A, minimum sugar concentration required to inhibit agglutination was taken. All lectin and sugar solutions were made in 6 mM potassium phosphate buffer, pH 7.4 containing 0.9% NaCl. In all cases 0.05 M boric acid (solution made in the phosphate buffer as mentioned above) was found to be sufficient to get positive agglutination. The controls for the sugar, borate and the buffer were also run and did not show positive agglutination.

Lectins (μg)	Sugar (M)	Borate (M)	Agglutination results
(A) Galactose Specific Lectins			
Peanut agglutinin	D(+) Galactose		
50	0.00	0.00	+
50	0.05	0.00	-
50	0.05	0.05	+
Soybean agglutinin	D(+) Galactose		
25	0.00	0.00	+
25	0.1	0.00	-
25	0.1	0.05	+
Jack fruit seed agglutinin	D(+) Galactose		
25	0.00	0.00	+
25	0.15	0.00	-
25	0.15	0.05	+
(B) N-acetyl glucosamine specific lectin			
Wheat germ agglutinin	N-acetyl D-glucosamine		
25	0.00	0.00	+
25	0.15	0.00	-
25	0.15	0.05	+
(C) Glucose specific lectin			
Concanavalin A	D-glucose		
10	0.00	0.00	+
10	0.05	0.00	-
10	0.05	0.05	+

+ ; positive agglutination
- ; no agglutination

effluent fractions of lectins obtained during their purification by affinity procedures since it enabled us to assay individual fractions for the lectin activity without dialysing the sugar.

Finally, we have also found this method particularly valuable during our work on chemical modification of lectins when the reactions are generally carried out in presence of specific sugars in order to get products with higher retention of lectin activity (Kamra and Gupta, 1988).

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