INHIBITION BY SACCHARIDES OF THE AGGLUTINATION OF PERIODATE-TREATED ERYTHROCYTES BY HORSE SERUM

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1. Introduction

Human erythrocytes after treatment with sodium periodate ($NaIO_4$) are rendered agglutinable by all adult human sera tested [1, 2], and by sera from other mammalian species [3]. The agglutinin for $NaIO_4$-treated erythrocytes (P-agglutinin) was characterized as a 19 S macroglobulin [4]. Further studies [1, 2] have shown that treatment of human erythrocytes with $NaIO_4$ modified their antigenic properties. We report here on the inhibition by specific saccharides of the agglutination of $NaIO_4$-treated erythrocytes by the P-agglutinin present in horse serum.

2. Materials and methods

2.1. *Materials*

Horse serum was obtained from Grand Island Biological Co., USA, and was incubated for 30 min at 56° prior to its use. Sodium periodate, analytical reagent, was obtained from BDH Chemicals Ltd., England. Saccharides were obtained from Pfanstiehl Laboratories, Inc., Illinois.

2.2. *Treatment of erythrocytes with $NaIO_4$*

Washed human or rat erythrocytes were suspended (16×10^6/ml) in phosphate buffered saline (PBS) [5], containing $NaIO_4$ (4×10^{-4} M) and incubated for 10 min at room temp. The cells were then centrifuged and washed 3 times with 100 volumes of PBS and suspended in the same buffer.

2.3. *Agglutination assay*

The agglutination assay system contained in a total volume of 0.6 ml: 0.4 ml of $NaIO_4$-treated erythrocytes (12.5×10^6/ml in PBS), 0.1 ml of horse serum diluted in PBS and 0.1 ml of saccharide (200 mg/ml in PBS). The extent of the agglutination was estimated by determining the percentage of single cells remaining in the incubation mixture after incubation at room temp. for 2 hr and 10 hr.

3. Results and discussion

The effect of several saccharides on the agglutination of human erythrocytes by horse serum is shown in table 1. The results obtained showed that N-acetyl-D-glucosamine and N-acetyl-D-galactosamine, markedly inhibited the agglutination of $NaIO_4$-treated erythrocytes by horse serum. A much lesser degree of inhibition was obtained with some of the other saccharides tested. Similar results were obtained when $NaIO_4$-treated human erythrocytes of type O, human erythrocytes of type A or rat erythrocytes were used.

The site of action of $NaIO_4$ on the cell membrane is not known. However, it is most plausible that $NaIO_4$ exerts its effect by oxidizing carbohydrate moieties in the cell membrane. It is thus possible that in the chemically modified membrane new sites are exposed which are capable of interacting with the P-agglutinin present in horse serum. N-acetyl-D-glucosamine and N-acetyl-D-galactosamine, most likely, inhibit the agglutination of $NaIO_4$-treated erythrocytes as a result of a competitive inhibition reaction in which the above mentioned saccharides and the membrane sites exposed by $NaIO_4$-treatment compete for the binding sites of the P-agglutinin. Uhlenbruck and Wintzer [6] recently reported the appearance of new

Table 1
Effect of saccharides on the agglutination of $NaIO_4$-treated human erythrocytes by horse serum.

Saccharide added	Percentage of single cells remaining after 2 hr final dilution of horse serum			10 hr		
	1:40	1:80	1:160	1:40	1:80	1:160
None	4	15	23	1	4	12
D-Glucose	4	25	29	2	7	12
D-Galactose	9	10	34	3	3	19
D-Mannose	14	29	31	6	9	9
L-Fucose	13	32	49	2	7	6
Methyl-α-D-mannopyranoside	3	16	57	0	4	32
N-Acetyl-D-glucosamine	43	92	98	19	87	96
N-Acetyl-D-galactosamine	25	94	95	–	85	–

Agglutination assays were performed as described in Materials and methods. $NaIO_4$-treated human erythrocytes of type A were used.

terminal sugars in erythrocytes after enzymatic removal of neuraminic acid. It is pertinent to note that treatment of erythrocytes with neuraminidase, similarly to treatment with $NaIO_4$, renders the cells panagglutinable [7]. However, the sites exposed by the two different treatments can be differentiated serologically [1, 2].

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