HAEMAGGLUTINATING ACTIVITY OF LEUCOAGGLUTININ AFTER NEURAMINIDASE TREATMENT OF HUMAN ERYTHROCYTES

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1. Introduction

Leucoagglutinin (also named L-PHA) is the isolectin of PHA (phytohaemagglutinin from *Phaseolus vulgaris* – red kidney bean) practically devoid of haemagglutinating activity, but with high leucoagglutinating power and strong lymphocyte stimulating activity [1]. The high haemagglutinating activity of PHA is concentrated in a second fraction (H-PHA) which is a mixture of isolectins with both mitogenic and red cell-agglutinating activities [2]. The molecule of leucoagglutinin is formed by four identical subunits which interact with glycidic receptors on lymphocytes [3,4]. The binding is specifically inhibited by *N*-acetylgalactosamine [5,6]. Leucoagglutinin interacts with human Tamm-Horsfall glycoprotein (T-H): the precipitin reaction is similar to a typical antigen–antibody reaction and is inhibited only by *N*-acetylgalactosamine [7]. After pronase digestion of T-H a glycopeptide can be isolated [8] which behaves as a powerful inhibitory hapten both in the precipitin reaction of T-H with leucoagglutinin and in lymphocyte transformation induced by leucoagglutinin [9].

Here a haemagglutinating activity of leucoagglutinin is shown to appear after neuraminidase treatment of human erythrocytes. The specificity of desialylated receptors for leucoagglutinin was investigated by the hapten-inhibition test of haemagglutination.

2. Experimental

2.1. *Materials*

Leucoagglutinin was from Pharmacia or was purified from commercial PHA as in [7]. T-H was prepared from human pooled urine according to [10]. Pronase digestion of T-H and purification of glycopeptide (T-H glycopeptide) were performed as detailed in [9]. Monosaccharides were from Sigma and neuraminidase from Behringwerke (1 U/ml).

2.2. *Neuraminidase treatment of human erythrocytes*

Human erythrocytes were washed 4 times with PBS (20 mM sodium phosphate buffer (pH 7.4), containing 0.14 M NaCl). Cells (2×10^8/ml) in PBS containing 1 mM $CaCl_2$ were incubated with neuraminidase (0.015 U/ml) at 37°C with shaking. At the indicated times incubations were stopped by diluting cell suspensions with 50 vol. cold PBS. The neuraminidase-treated erythrocytes were immediately centrifuged and washed 4 times with PBS.

2.3. *Haemagglutination assay*

Haemagglutination tests were performed in U-shaped Greiner microtitre plates. Each well contained in 100 μl final vol. serial dilutions of leucoagglutinin and 50 μl untreated or treated erythrocyte suspensions (1.2%, v/v). When the effect of glycidic haptens was tested, they were added to the wells just before erythrocytes. All solutions and suspensions were in PBS containing bovine serum albumin at 15 μg/ml. Haemagglutination was evaluated visually after 3 h at room temperature. The concentration of leucoagglutinin was determined by measuring the absorbancy at 280 nm ($A_{1\,cm}^{1\%}$ 11.4). The minimal amount of leucoagglutinin required to agglutinate is defined as one unit of haemagglutinating activity (HU).

3. Results and discussion

Fig.1 reports the enhancement of haemagglutinating activity of leucoagglutinin after neuraminidase

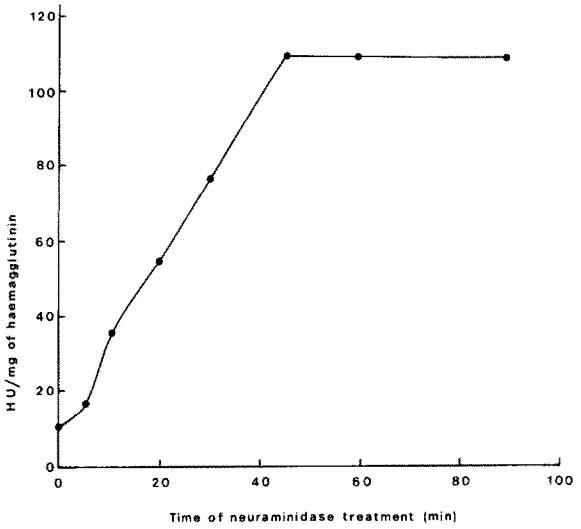

Fig.1. The effect of neuraminidase treatment of human erythrocytes on the haemagglutinating activity of leucoagglutinin. The erythrocytes used in the present experiment were group O. The experimental conditions are in section 2.

Table 1
Effect of glycidic haptens on the haemagglutination of neuraminidase-treated human erythrocytes by leucoagglutinin

Glycidic hapten	Conc.	Agglutination
None	–	+
T-H glycopeptide	3.2 μM	–
N-Acetyl-D-galactosamine	25 mM	–
N-Acetyl-D-glucosamine	100 mM	+
N-Acetyl-D-mannosamine	100 mM	+
D-Galactose	100 mM	+
D-Glucose	100 mM	+
D-Mannose	100 mM	+
L-Fucose	100 mM	+
1-*O*-Methyl-α-D-galactoside	100 mM	+
1-*O*-Methyl-β-D-galactoside	100 mM	+
1-*O*-Methyl-α-D-glucoside	100 mM	+
1-*O*-Methyl-β-D-glucoside	100 mM	+
1-*O*-Methyl-α-D-mannoside	100 mM	+

Human erythrocytes (group O) were treated with neuraminidase for 45 min. Leucoagglutinin was added at 2 HU/100 μl (final volume in each well). The concentration of T-H glycopeptide was calculated assuming mol. wt 4800

treatment of human erythrocytes. The time course of neuraminidase treatment shows a very rapid exposure of leucoagglutinin receptors as assayed by the haemagglutination test. After 45 min all receptors were unmasked. No differences were found with erythrocytes from the 4 types of the ABO system. The identical agglutination before and after neuraminidase treatment of type A compared to types O and B indicates that the terminal *N*-acetylgalactosamine of the A blood group determinant is not involved in the binding site for leucoagglutinin.

The results of glycidic hapten-inhibition tests of haemagglutination are shown in table 1. The only monosaccharide with inhibitory activity was *N*-acetylgalactosamine, active at 25 mM: T-H–glycopeptide resulted in molar terms ~10 000-times more inhibitory than *N*-acetylgalactosamine. The same ratio in the inhibitory activity of the two haptens was found in the precipitin reaction between T-H and leucoagglutinin [9]. These data strongly suggest that an identical oligosaccharide structure is present on the surface of neuraminidase-treated human erythrocytes and in the glyco-moiety of T-H. It is well known that many lectins are able to interact with surface cell receptors after deletion of *N*-acetylneuraminic acid. Neuraminidase-treated erythrocytes and lymphocytes are agglutinated by peanut agglutinin [11] and neuraminidase-treated mouse spleen cells by soybean agglutinin [12] because galactosyl sites become exposed. These results show that removal of *N*-acetylneuraminic acid from human erythrocytes unmasks *N*-acetylgalactosamine residues.

The carbohydrate analysis of T-H glycopeptide gives 2.9% *N*-acetylgalactosamine measured by the colorimetric method in [9], and 3.9% as measured by gas–liquid chromatography (unpublished). This means 1 residue/molecule, assuming mol. wt 4800 for T-H glycopeptide (R. D. Marshall, personal communication). In spite of its low content in *N*-acetylgalactosamine, the T-H glycopeptide shows the same inhibitory activity as the monosaccharide, at a concentration 4 log lower. This suggests that the affinity of the ligand for leucoagglutinin is strongly affected by a specific position of *N*-acetylgalactosamine in the oligosaccharide structure.

The high sensitivity of the haemagglutination test and the rapidity of its performance may by utilized in studies on the binding of leucoagglutinin to the cell receptors and in investigating the complexity of the carbohydrate specificity of this lectin.

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