CARBOHYDRATE COMPONENTS OF THE SURFACE OF THE THYROGLOBULIN MOLECULE

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To obtain information on the carbohydrate components of thyroglobulin, the method of inhibition of antithyroid precipitating activity of phytoprecipitin from the seeds of Pisum sativum L. by saccharides was used. The results showed that the determinants of thyroglobulin which interact with phytoprecipitin are oligosaccharides; the terminal sugar of these determinants is N-acetylglucosamine or D-mannose, or both these monosaccharides; the next monosaccharide in the oligosaccharide chain is probably fructose.

The study of inhibition of the agglutination reaction observed between phytohemagglutinins and red blood cells by saccharides has yielded much important information on the composition and structure of the antigenic determinants of certain antigens. For instance, it has been found by this method that antigenic determinants on the surface of red cells and reacting with lectins include carbohydrates, and the specificity of the antigens is determined by the order of arrangement of the sugars [5, 6, 10].

Previously one of the writers (S.I.A.) [1, 3] reported the discovery of an antithyroid phytoprecipitin from seeds of Pisum sativum L., reacting specifically with extract from normal and pathological human thyroid glands.

In the present investigation the method of inhibition of serological activity of lectins by saccharides was used to obtain information on the composition and structure of the determinants of thyroglobulin to which the specificity of the antithyroid phytoprecipitin is directed.

EXPERIMENTAL METHOD

Extract from Pisum sativum was obtained as described previously [3]. To precipitate the ballast proteins the extract was cooled to 6°C, and cold 96° ethanol was added with mixing in the proportion of 5:1 (by volume). Next day the extract was centrifuged for 15 min at 2000 rpm, the residue was removed, and 2 N NaOH solution was added drop by drop to the supernatant until the pH of the solution was 7.5. The precipitate thrown down during keeping overnight at 6°C was removed by centrifugation at 2000 rpm for 15 min. The resulting transparent extract, pale yellow in color, was lyophilized. A solution of the lyophilized powder in distilled water, added in a volume equal to the original volume of the extract, was used in the precipitation test.

The inhibitory action of the following mono- and oligosaccharides was studied: D-galactose, D-xylose, D-fructose, D-ribose (East Germany), L-rhamnose, D-talose, cellobiose, L-sorbose, (Czechoslovakia), D-glucose (Poland), sucrose, raffinose (USSR), N-acetylglucosamine, and D-mannose.

Solutions (1 M) of the sugars were diluted with physiological saline in the ratio 1:1, and later to a dilution of 1:128. Next, two drops of sugar solution of the corresponding concentrations were treated with two drops of phytoprecipitin solution. The mixture was incubated for 20 h at 18°C.

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TABLE 1. Inhibition of Antithyroid Phytoprecipitin from P. sativum by Sugars

Substance	Intensity of precipitation with sugar in dilutions of							
	undiluted	1:2	1:4	1:8	1:16	1:32	1:64	1:128
Physiological saline (control)	+++	+++	+++	+++	+++	+++	+++	+++
N-Acetylglucosamine	0	0	0	0	+	++	+++	+++
D-Glucose	0	0	+	++	++	+++	+++	+++
D-Galactose	+++	+++	+++	+++	+++	+++	+++	+++
D-Xylose	0	0	+	+	++	+++	+++	+++
D-Mannose	0	0	0	0	+	++	+++	+++
Raffinose	+++	+++	+++	+++	+++	+++	+++	+++
D-Ribose	+++	+++	+++	+++	+++	+++	+++	+ ++
L-Rhamnose	+++	+++	+++	+++	+++	+++	+++	+++
Cellobiose	0	+	+	++	++	+++	+++	+++
D-Talose	+++	+++	+++	+++	+++	+++	+++	+++
L-Sorbose	0	0	+	++	+++	+++	+++	++ +
D-Fructose	0	+	+	++	+++	+++	+++	+++
Sucrose	0	0	0	+	+	++	+++	+++

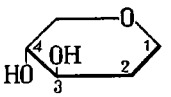
The antithyroid activity of the adsorbed extract was tested in Ouchterlony's gel-diffusion test in the micromodification of Gusev and Tsvetkov [2], using as the antigen an extract from a human thyroid gland prepared by the method described previously [1].

EXPERIMENTAL RESULTS

The experimental results are shown in Table 1. N-acetylglucosamine and D-mannose were clearly the most effective inhibitors of the antithyroid phytoprecipitin. The precipitation reaction was inhibited to a lesser degree by sucrose, D-glucose, D-xylose, and L-sorbose, and to an even lesser degree by D-fructose and cellobiose. The remaining sugars were inactive.

Thyroglobulin is known to be a glucoprotein. It includes in its composition mannose, N-acetylglucosamine, and sialic acid [4, 7, 8]. The results of the present experiments showing inhibition of the antithyroid activity of extract from P. sativum by N-acetylglucosamine and D-mannose are evidence that the mannose and N-acetylglucosamine are components of the thyroglobulin determinant, with which the antithyroid phytoprecipitin reacts.

According to the classification suggested by Mäkelä [9], the sugars inhibiting the hemagglutinating activity of the phytohemagglutins are subdivided into four groups depending on the position of the hydroxyl group at the 3rd and 4th carbon atoms of the pyranose ring. The results of the present investigations showed that the sugars inhibiting the antithyroid precipitating activity of extract from P. sativum, as well as the sugars inhibiting its hemagglutinating activity, belong to Mäkelä's group 3, characterized by the following configuration of the substituents at the 3rd and 4th carbon atoms:



Paulova et al. [11] consider that the antigenic determinants of red blood cells and of some polysaccharides interacting with lectin from P. sativum are identical in the structure of their sugars. The results now obtained support this hypothesis, and also indicate that this is evidently true also as regards the corresponding determinants of thyroglobulin.

It is interesting to note that sucrose is a more active inhibitor of antithyroid activity of phytoprecipitin than cellobiose, and that N-acetylglucosamine and D-mannose are better inhibitors than glucose and fructose. Finally, sucrose which, as we know, consists of glucose and fructose (2-α-glucopyranosido-β-fructofuranoside) is a better inhibitor than glucose and fructose. This indicates that the determinants of thyroglobulin which interact with phytoprecipitin are oligosaccharides; the terminal sugar of these determinants is N-acetylglucosamine or D-mannose, or both monosaccharides; the next monosaccharide in the chain of this oligosaccharide is probably fructose. The authors have previously shown that extracts from the seeds of plants belonging to the genera Vicia and Lathyrus also contain phytoprecipitins which interact specifically with the thyroid gland [3]. Boyd [5] has shown that the hemagglutination reaction between red cells and lectins from Vicia and Lathyrus is inhibited by sugars of Mäkelä's group 3. It can therefore be

considered that the phytoprecipitin from Vicia and Lathyrus interacts most probably with the same determinants of thyroglobulin as the antithyroid phytoprecipitin from P. sativum.

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