The role of carbohydrates in the radioimmunoassay of human low-molecular-mass kininogen

Ursula Turpeinen* and Tytti Kärkkäinen

Department of Biochemistry, University of Helsinki, Unioninkatu 35, 00170 Helsinki, Finland

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The immunoreactivity of human low-molecular-mass kininogen from Cohn plasma fraction IV was investigated after deglycosylations and carbohydrate modifications by radioimmunoassay using the conformation-specific antiserum. Removal of all sialic acids, 44% of amino sugars and 63% of neutral sugars did not alter the immunoreactivity of the protein but the periodate-treated concanavalin A fractions showed strikingly diminished immunoreactivity. A conformational change could account for the observed effect of periodate on the decreased reactivity of the protein in radioimmunoassay. Externally added carbohydrates had no effect on immunoreactivity. The results suggest that the carbohydrate part of kininogen is not involved in the immunoreactivity although it accounts for the observed lectin-binding heterogeneity.

Carbohydrate *Glycoprotein* *Kininogen* *Radioimmunoassay* *Lectin* *Exoglycosidase*

1. INTRODUCTION

Two types of kininogens, a high-M_r (M_r 120000) and a low-M_r (M_r 50000–70000) form exist in human plasma [1]. Also, a kinin-free low-M_r kininogen (M_r 60000) from Cohn plasma fraction IV, H_C antigen, has been isolated and characterized [2]. Both native kininogens are glycoproteins and the precursors of the vasoactive kinins. The charge heterogeneities of the 2 native plasma kininogens have been investigated [3,4]. The low-M_r kininogen occurs in several immunoreactive isoelectric forms with the main component at pI 4.5–4.6 and that of high-M_r kininogen at pI 4.7. The different isoelectric pattern of H_C antigen with a low pI of 4.2–4.4 is suggested to result from the structural variations in the carbohydrate part of the molecule [2,5]. Two different carbohydrate structures of H_C antigen obtained after concanavalin A (Con A) chromatography show, however, identical isoelectric patterns [6]. This indicates that the lectin-binding heterogeneity is not charge dependent.

Here we investigate the role of carbohydrates in the immunoreactivity of H_C antigen by removing or modifying the sugar residues. Exoglycosidase treatments, addition of carbohydrates and chemical modifications are performed. The antigenicity of the protein is measured by radioimmunoassay (RIA) using antisera against the conformational determinants of H_C antigen [7].

2. MATERIALS AND METHODS

2.1. *Preparation of H_C antigen*

H_C antigen was purified from freshly precipitated Cohn plasma fraction IV [2]. Immunological purity was achieved with antibody-specific polymers by removing impurities of α_2HS-glycoprotein, ceruloplasmin and albumin [8]. Quantitative determinations of immunoreactive kininogen were performed by single radial immunodiffusion [9,10]. Protein was measured by A_{280} using $A_{280}^{1\%} = 7$ for pure kininogen.

* To whom correspondence should be addressed at: Helsinki University Central Hospital, Meilahti Hospital Laboratory, Haartmaninkatu 4, 00290 Helsinki, Finland

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2.2. *Preparation of antiserum*

Antiserum against H_C antigen was prepared in rabbits using the immunologically pure antigen containing the conformation-dependent determinants of the native kininogen [7]. The association constant of this antiserum against the native kininogen was $3.8 \times 10^9\ M^{-1}$.

2.3. *RIA*

Radiolabelling of H_C antigen to a specific activity of 60 μCi/μg was performed according to Greenwood et al. [11] using chloramine T. Free $Na^{125}I$ (IMS 30, Amersham) was removed by gel filtration on Sephadex G-25 using 50 mM sodium phosphate buffer, pH 7.5, and the column was washed with 1% bovine albumin (RIA grade, Sigma) before use. The radioiodinated protein was diluted 1:10 in 50 mM sodium phosphate buffer, pH 7.5, containing 0.25% bovine albumin, 0.15 M NaCl and 0.01% NaN_3 and stored at −70°C. The antiserum was diluted 1:20000 in the same buffer for RIA [7,12].

2.4. *Affinity chromatography on lectins*

Columns of Con A-Sepharose 4B were used as in [5]. *Ricinus communis* agglutinin (RCA_1) was isolated from castor beans, coupled to CNBr-activated Sepharose 4B and the affinity chromatography run as described [6].

2.5. *Exoglycosidase treatments of kininogen*

The digestion of 950 μg immunoreactive H_C antigen was performed using 1.5 units neuraminidase (*Clostridium perfringens*, type VI, Sigma), 1.0 unit β-*N*-acetylglucosaminidase (jack beans, Sigma), 0.8 unit α-mannosidase (jack beans, type III, Sigma) and 0.8 unit β-galactosidase (*Aspergillus niger*, grade V, Sigma) in 1 ml of 50 mM sodium citrate buffer, pH 5.0, at 37°C for 72 h. The released sialic acids, neutral sugars and amino sugars were quantitated [13–15] after dialysis. The neuraminidase treatment of 3.0 mg H_C antigen was performed in 0.1 M acetate buffer, pH 5.0, with 3 units enzyme for 2 h at 37°C.

2.6. *The added monosaccharides in RIA*

1.8 ng *N*-acetylneuraminic acid (type IV, Sigma), 1.0 ng D-galactose (G-0750, Sigma) and 0.5 ng D-mannose (M-4625, Sigma) were separately added to a solution of 41 ng H_C antigen in 200 μl of 50 mM sodium phosphate buffer, pH 7.5, 0.2% gelatine, 0.15 M NaCl and 0.01% NaN_3 and the solutions were analyzed in RIA.

2.7. *The periodate treatment*

The Con A nonreactive and reactive fractions (200 μg protein from both pools) were incubated in 3 ml of 20 mM sodium metaperiodate at 4°C in the dark for 6 h and dialyzed against 10 mM sodium phosphate, 0.15 M NaCl, pH 7.0, overnight.

3. RESULTS AND DISCUSSION

The Con A reactive and non-reactive fractions of H_C antigen previously showed identical dose-response curves in RIA [6]. Here we treated both Con A fractions with periodate and applied them to RIA. Con A is known to distinguish various branching patterns of mannose [16,17] and the periodate treatment of glycoproteins results in selective modification of sialic acids [18]. Immunoreactivity of the periodate-treated Con A reactive and non-reactive fractions was reduced to 34 and 17% of the untreated H_C antigen when tested in RIA with the conformationally dependent antiserum (fig.1). This indicates that the im-

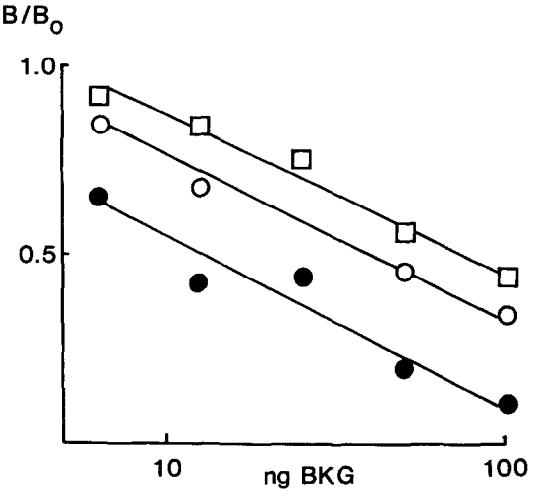

Fig.1. Dose-response curves for native H_C antigen (●) and the Con A reactive (○) and non-reactive (□) fractions of H_C antigen after periodate treatment using conformationally dependent antiserum. BKG, H_C antigen.

munoreactivity of H_C antigen is greatly influenced by the modification of sialic acids.

H_C antigen was next incubated with neuraminidase and the product was separated by *R. communis* lectin. The *Ricinus*-reactive fraction was submitted to RIA. By using the reactive component we obtained a fraction free of terminal sialic acids since *Ricinus* lectin is known to be specific for terminal galactose residues [19]. The immunoreactivity of H_C antigen was not influenced by the complete removal of sialic acids as parallel and identical dose-response curves were obtained in RIA (fig.2).

The decrease in immunoreactivity seen after periodate treatment (fig.1), however, seems to be independent of sialic acids since no deterioration of immunoreactivity was detected after treatment with neuraminidase (fig.2). This supports a concept of a conformational change in both Con A fractions caused by the periodate treatment.

The influence of the total removal of sialic acids and partial removal of amino sugars and neutral sugars on immunoreactivity was investigated by using a mixture of exoglycosidases. All sialic acids but only 44% of amino sugars and 63% of neutral sugars could be removed. The exoglycosidases remove terminal non-reducing monosaccharides but their activity on intact glycoproteins is more limited [20]. Some of the carbohydrates of H_C antigen are apparently masked by the polypeptide core and are resistant to cleavage. However, the native and enzyme-treated proteins showed identical dose-response curves in RIA (fig.3). This indicates that the antigenic sites of H_C antigen are located in the polypeptide part of the molecule and that sialic acids and 44% of amino sugars and 63% of neutral sugars do not affect the antigenicity of the protein. It cannot, however, be excluded that the remaining carbohydrate side chains play a role in the conformation of the protein and in its immunoreactivity.

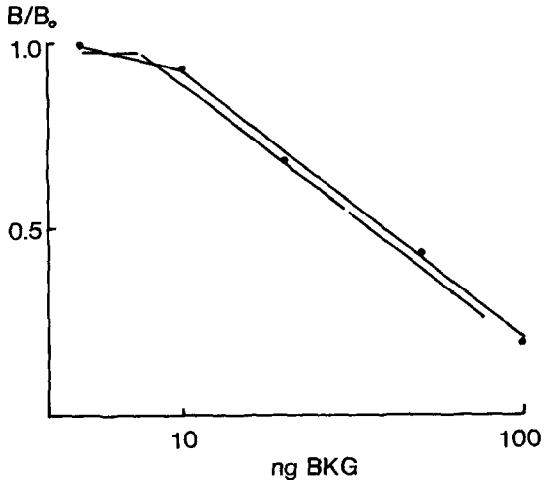

Fig.2. Displacement curves for the native (●) and the desialylated (○) H_C antigen obtained after affinity chromatography on *R. communis* lectin.

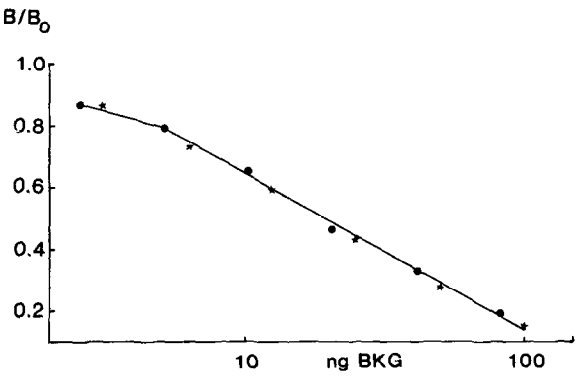

Fig.3. Immunoreactivity of the native (●) and partially deglycosylated (*) H_C antigen. 950 μg immunoreactive H_C antigen was treated with a mixture of exoglycosidases.

The involvement of externally added monosaccharides in the immunoreactivity of H_C antigen was further studied by RIA. The added amounts of sugars were those normally present in the intact protein [6]. However, no effect on immunoreactivity was seen (fig.4).

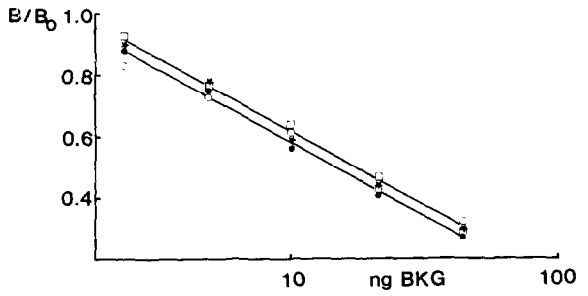

Fig.4. Dose-response curves for the native H_C antigen (●) and after addition of sialic acid (*), galactose (○) and mannose (□).

In conclusion, H_C antigen had been previously shown to have an extensive charge heterogeneity [2] which was suggested to be due to the variations of the carbohydrate part of the molecule. This study indicates that sialic acids are not essential for the antigen-antibody reaction of H_C antigen. A large portion of amino sugars and neutral sugars are neither involved in the immunoreactivity of the protein although they seem to be the main cause of the previously found charge heterogeneity. Our results confirm that the immunoreactivity of H_C antigen is mainly determined by the protein part of the molecule.

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