ABSENCE OF INTERACTION BETWEEN CONCANAVALIN A AND HEPARIN OR HEPARIN SULFATE

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1. Introduction

Concanavalin A, the phytohemagglutinin of jack bean (*Canavalia ensiformis*) precipitates various branched glycans by interacting with their terminal, non-reducing units [1].

Inhibition studies with the system dextran-concanavalin A have indicated that the specificity of the protein is directed primarily toward the C-3, C-4 and C-6 hydroxyl groups of D-mannopyranosyl or D-glucopyranosyl structures possessing an α-configuration at C-1. Modifications of the molecules at these sites cause disappearance of inhibitory activity. The hydroxyl group at C-2 seems to be also involved in the interaction, as indicated by the higher inhibitory activity of D-mannose in respect to D-glucose and by the lack of inhibitory activity of 2-acetamido-2-deoxy-D-mannose. 2-Acetamido-2-deoxy-D-glucose, however, has an inhibitory activity essentially similar to that of D-glucose, while the charged 2-amino-2-deoxy-D-glucose has none [1, 2].

On the basis of this information, one would consider probable interaction between concanavalin A and glycosaminoglycans which contain, and may terminate, with non-reducing 2-acetamido-2-deoxy-D-glucose units possessing α-configuration at position 1. Both heparin and heparan sulfate contain 2-acetamido-2-deoxy-D-glucose and 2-sulfamido-2-deoxy-D-glucose-6-sulfate [3]. The former amino sugar has been reported to be more abundant in the interior of the heparan sulfate or at the non-reducing terminus [4]. Both polymers have α-(1→4)-glycosidic linkages [3, 5] and have been suspected of being branched rather than linear structures [6, 7].

Two reports have appeared in the literature describing the interaction of concanavalin A with heparin. The first one [8] stated that sodium heparinate had 50% more precipitating ability than a standard preparation of normal human liver glycogen. This ability to interact with concanavalin A was apparently unrelated to the sulfate groups of heparin, since it did not disappear upon desulfation and acetylation of the polymer nor when the polymer was partially deacetylated to give *N*-acetyl desulfated heparin. The second report [9] stated that commercially obtained heparin had 35% of the affinity of glycogen for concanavalin A and that the interaction could be inhibited completely by 1 M NaCl and decreased 50% by 0.1 M glucose. These data imply that the interaction between heparin and concanavalin A depends in part on electrostatic forces, a conclusion which is in contrast with the results obtained by Cifonelli et al. [8] using desulfated heparin.

We have undertaken these experiments in order to investigate the possibility of identifying heparin and heparan sulfate in biological fluids, using concanavalin A as a specific reagent. Under the experimental conditions employed, no interaction between concanavalin A and these glycosaminoglycans was detected.

2. Materials and methods

Concanavalin A was prepared from 2 kg whole jack beans (Sigma Chemical Co., St. Louis, Mo.) according to Doyle's technique [9] with the following differences. After the elution of concanavalin A from Sephadex G-200 (Pharmacia Fine Chemicals, Piscataway, N.J., USA) with 1.0 M glucose, dialysis of the eluate produced a large amount of precipitate which was removed by centrifugation and discarded. The clear supernatant was dialyzed exhaustively and then lyophilized. Upon solution in water, an insoluble sediment was again removed by centrifugation and the clear supernatant was lyophilized. The dry material obtained (1.37 g) was used for the experiments described.

Rat liver glycogen was prepared with the method of Stetten et al. [10]. Rabbit liver glycogen was purchased from Sigma Chemical Co. (Type III, No. G-8876, Lot 99B-0700). The following preparations of glycosaminoglycans were used:

1) Heparin, sodium salt (Calbiochem Lot 63525, B grade). On analyses, it had hexuronic acid 32.5%, sulfur 9.10%, 2-sulfamido-2-deoxy-D-glucose 26.7%, neutral sugars (anthrone) 8%. The molecular weight, determined by sedimentation equilibrium, was 8,240.

2) Heparin, potassium salt (gift of Dr. D.D.Dziewiatkowski, University of Michigan, Ann Arbor, Mich.) had carbon 21.5%, nitrogen 1.7%, sulfur 9.84%, ash 46.5%, neutral sugars 5.8%, $[\alpha]_D^{21} + 40°$, molecular weight 11,000 ± 1,000.

3) Heparin (from hog mucosal tissues, fractionated by cetylpyridinium chloride precipitation; gift of Dr. J.A.Cifonelli, University of Chicago, Ill.) had nitrogen 2.6% hexuronic acid 38.7%, hexosamine 23.8%, neutral sugars 8.6% and the following molar ratios to glucosamine taken as 1.00: sulfate 2.33, galactosamine 0.001, 2-sulfamido-2-deoxy-D-glucose 0.89; $[\alpha]_D^{24} + 52°$, molecular weight [11] 11,000.

4) Heparan sulfate, calcium salt (from beef lungs, purified by fractionation on Dowex 1 column; gift of Dr. J.A.Cifonelli) had nitrogen 2.5%, hexuronic

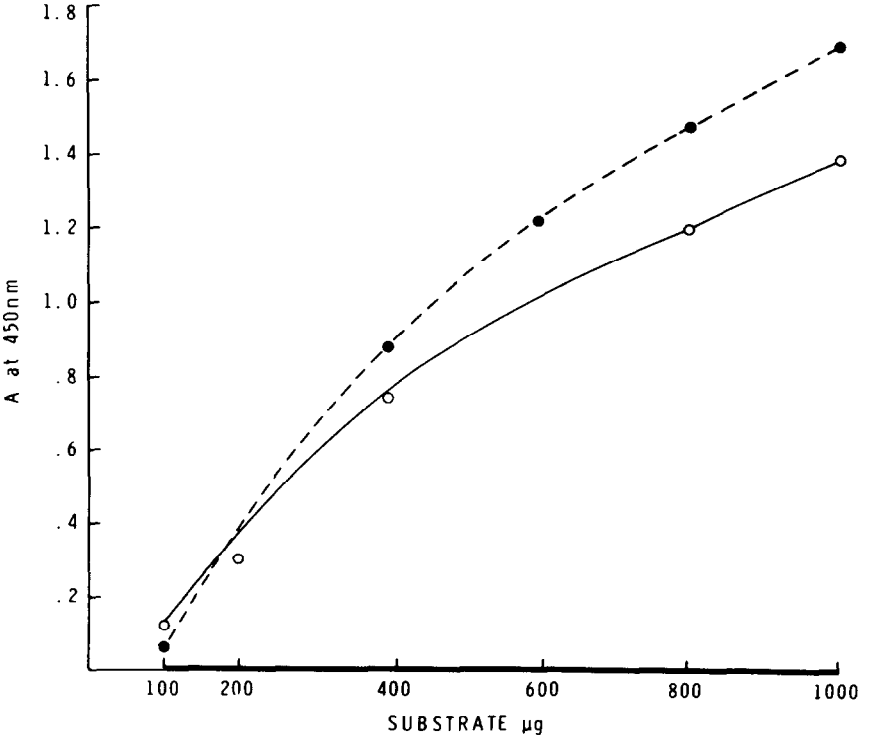

Fig. 1. Interaction between concanavalin A and rat liver glycogen (——), rabbit liver glycogen (– – –) and various preparations of heparin and heparan sulfate (——).

acid 44.1%, hexosamine 24.6% and the following molar ratios to glucosamine taken as 1.00: sulfate 0.99, galactosamine 0.002, 2-sulfamido-2-deoxy-D-glucose 0.51; $[\alpha]_D^{24} + 73°$.

5) Heparan sulfate (gift of Dr. K.Von Berlepsch, Hoffman-Laroche, Basel, Switzerland, Lot Ro 1-2232/754).

Each of these preparations was dissolved in pH 5.50 acetate buffer 0.05 M, 0.10 M NaCl [9], to a concentration of 1 mg/ml. Concanavalin A was also dissolved in the buffer to a concentration of 4 mg/ml. Its reactivity with the various glycosaminoglycans was measured with the method of Doyle et al. [9], adding 1 ml of the protein solution to decreasing amounts of each glycosaminoglycan in 1 ml of the buffer.

3. Results and discussion

The results reported in fig. 1 indicate that both preparations of glycogen reacted with concanavalin A but none of the glycosaminoglycans tested did so. Thus, our results disagree with those of Cifonelli et al. [8] and Doyle et al. [9].

One can only speculate on the reasons for these differences. Unfortunately, no analytical data of the heparin preparations used in the previous experiments were available, so that it is impossible to compare the relative purity of the various preparations or their physical properties. However, the fact that the heparin preparation used by Doyle et al. [9] had an anthrone hexose value of 15.2% may indicate a possible contamination with other glycans. Our preparations of heparin had satisfactory analytical data, molecular weights and biological activities. Thus, it is not likely that the absence of interaction with concanavalin A might have been due to factors related to their size or shape [12]. Rather, it is possible that despite the presence in these glycosaminoglycans of 2-acetamido-2-deoxy-D-glucose residues, these might have been too scarce on a molar basis or in the wrong site to assure a sufficient number of reactive sites for the binding with concanavalin A [12]. While the interaction of heparin and concanavalin A, as reported by Cifonelli et al. [8] did not seem to depend on electrostatic forces, that described by Doyle et al. [9] was dependent on them and could be prevented by 1.0 M NaCl. One wonders, then, whether the presence of other protein, besides concanavalin A, in the Sephadex eluate might have been responsible for the interaction with heparin. The latter glycosaminoglycan is known for its ability to bind various proteins in absence of a sufficient concentration of counterions.

Acknowledgments

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