INTERACTION BETWEEN CONCANAVALIN A AND HEPARIN

R.J. DOYLE* and Tze-Jou KAN
Department of Microbiology, University of Louisville School of Medicine, Louisville, Kentucky 40201, USA

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1. Introduction

It has been known for many years that concanavalin A, the jack bean phytohemagglutinin, precipitates with polysaccharides [1]. However, only since the paper by Goldstein et al. [2] in 1965, has the concanavalin A–polysaccharide system been studied in detail. Branched glucans, fructans and mannans interact with concanavalin A through their non-reducing terminii [3]. One feature of concanavalin A–neutral polysaccharide complexes is their stability in wide concentrations of salts [4], thereby negating electrostatic effects in the interaction.

A report by Doyle et al. [5] demonstrated that concanavalin A would interact with heparin and showed that complex formation could be inhibited by either sodium chloride or D-glucose, the latter being a known inhibitor of concanavalin A–neutral polysaccharide interactions. Previously, Cifonelli et al. [6] reported an interaction between concanavalin A and heparin. More recently, DiFerrante and Hrgovcic [7] could find no evidence for complex formation between the phytohemagglutinin and heparin using the conditions of Doyle et al. [5]. These findings led us to re-examine the interaction between concanavalin A and heparin. In this report, it is shown that concanavalin A forms precipitin-like complexes with heparin and that the interaction is pH, salt and sugar sensitive.

* To whom correspondence should be addressed.

2. Materials and methods

Concanavalin A used in this study was prepared from finely ground jack beans by the standard Agrawal and Goldstein [8] procedure. Following initial purification, the concanavalin A was recycled to insure its purity.

Oyster glycogen was purchased from Nutritional Biochemicals, Cleveland, Ohio. Methyl α-D-mannopyranoside was purchased from Calbiochem, Los Angeles, Calif. Three heparin samples were used in this study: A) Heparin, Na salt, B grade, Calbiochem. Analyses showed that this sample contained 44% uronic acid (as glucuronic acid equivalents [9], 6.7% hexose (anthrone), 1.95% nitrogen [10], 10.0% sulfur [11] and 16.0% ash. B) Heparin, Na salt, grade I, Sigma, St. Louis, Mo. This preparation contained 42% uronic acid, 7.3% hexose, 1.85% nitrogen, 13.0% sulfur and 16.7% ash. C) Heparin, Li salt, grade IV, Sigma. Analyses of this sample gave 42% uronic acid, 6.8% hexose, 2.05% nitrogen, 13.0% sulfur and 10.1% ash. Within experimental error, the different heparin preparations gave identical results. Therefore, for purposes of brevity, only the data for the heparin-Li salt will be discussed.

The following procedure was followed to study concanavalin A–heparin precipitate formation: concanavalin A (1 ml at 2.0 mg/ml) and heparin (1.0 ml at 0.032–4.0 mg/ml) in deionized water were added to 2.0 ml of 0.10 M buffer solution. After 20 min, the turbidities were measured in 0.5 inch rounded cuvettes in a Bausch and Lomb Spectronic 20 colorimeter at 420 nm. Buffer solutions were prepared according to Gomori [12]. Acetate buffers

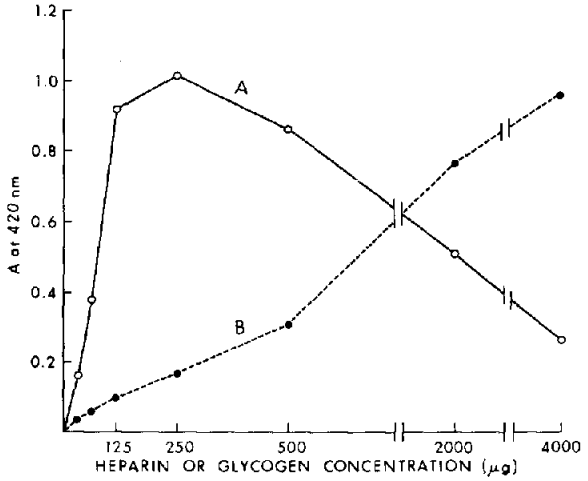

Fig. 1. The effects of heparin (A) and glycogen (B) concentration on precipitation with concanavalin A in 0.05 M acetate buffer, pH 5.4.

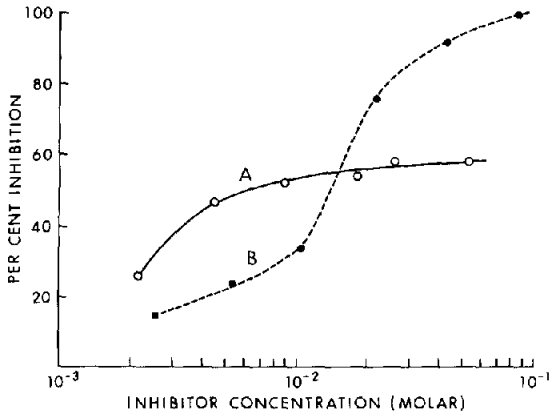

Fig. 2. Inhibition of the reaction between concanavalin A and heparin by methyl α-D-mannopyranoside (A) and sodium chloride (B).

were used between pH 3 and 5.6 and above pH 5.8 phosphate buffers were employed. Hydrogen ion concentrations were determined immediately following the turbidity measurements. In the experiments requiring added sodium chloride or methyl α-mannoside, the addition was made to buffer prior to concanavalin A and heparin.

3. Results and discussion

Because of its polyanionic character, heparin should be able to form electrostatic complexes with a variety of proteins. Several cases have been documented showing heparin–protein interactions [13, 14]. The concanavalin A–heparin system may be more complex. Concanavalin A and heparin were mixed at different pH values and the turbidities recorded. No interaction was observed above pH 5.8. A pH of 5.4 gave optimum turbidity. This contrasts with concanavalin A–neutral polysaccharide interactions in which the pH optimum is near neutrality [4]. Analyses of the precipitates for uronic acid and protein showed that the insoluble materials were concanavalin A–heparin complexes. Turbidity was proportional to the amount of heparin and concanavalin A in the precipitates.

The effect of heparin concentration is shown in figl 1. A classical precipitin-like reaction was obtained for each of the heparin samples in 0.05 M acetate buffer, pH 5.4. Based on the turbidimetric assay, 2.0 mg concanavalin A combined with approx. 0.25 mg heparin at the equivalence point. In contrast to heparin, the concanavalin A precipitin profile with glycogen is much broader (fig. 1) [15]. Qualitatively, the concentration dependence of the concanavalin A–heparin complex is similar to that of concanavalin A–dextran complexes [2].

The foregoing data firmly establish that concanavalin A and heparin interact to form visible complexes. The question to be answered is whether heparin and neutral polysaccharides bind to the same site(s) on the concanavalin A molecule. It is shown in fig. 2 that NaCl at concentrations approaching 0.1 M completely inhibits the concanavalin A–heparin interaction. This is consistent with the concept that the complex is mediated through ionic binding. If the interaction were dependent on the binding of sugar termini by concanavalin A, inhibition should be observed when methyl α-mannoside is present [3]. At 0.053 M, methyl α-mannoside gave approx. 58% inhibition (fig. 2) Much lower concentrations of methyl α-mannoside are required to completely inhibit concanavalin A–neutral glucan interactions [3].

In our earlier report [5], 0.1 M NaCl concentrations were used and a concanavalin A–heparin complex was observed. Using the same conditions, DiFerrante and Hrgovic [7] saw no precipitation. From the data presented in this paper, it is apparent that 0.1 M NaCl is near the threshold concentration for total inhibition of visible precipitate formation and that slight variations in ionic strength profoundly influence the interaction. Our previously used heparin preparation was not analyzed for ash. Commercial heparin preparations frequently contain salts. Thus, it is possible that contaminating salts would add to the inhibitory effects of exogenous ions.

The fact that methyl α-mannoside is a partial inhibitor of the concanavalin A–heparin complex presents an interesting situation. If the complex were completely dependent on electrostatic forces, methyl α-mannoside would not be expected to show competitive effects. We have shown (R.J. Doyle and R. Glew, unpublished results) that methyl α-mannoside induces a conformational change in concanavalin A. Thus, a ligand-induced conformational change in the protein may result in the masking of ionic sites involved in the interaction with heparin.

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