

THE POSITIVELY COOPERATIVE BINDING OF CONCAVALIN A TO CORN STARCH: THE ISOTHERM OF BINDING AND A MEASURE OF THE COOPERATIVITY

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ABSTRACT

The binding of concanavalin A to corn starch was investigated by fluorimetric assay. The extent of binding varied linearly with the mass of ligand, and followed a hyperbolic law with respect to the mass of starch. This led to an isotherm of binding: $r = 0.33A_oME_o^{-0.88}$, where r is the extent of binding, A_o is the mass of concanavalin A present (both bound and unbound), and M_o is the mass of starch. These results, and Scatchard plots of the data, showed the binding to be positively cooperative. The exponent of the M_o term was shown to be a measure of cooperativity. The binding was dependent on the ionic strength of the dispersion medium, and this indicated that the binding may have an electrostatic component.

INTRODUCTION

It has been observed that the specificity that lectins display in binding to macromolecules extends to large particles, such as starch grains¹, and to particulate, organized, biological systems, such as erythrocytes¹ and micro-organisms². In the latter systems, positive cooperativity and agglutination of the particles are common sequels to the binding¹.

In order to understand the mechanisms of both the binding of the lectins to the particles and the agglutination, a logical first step is to establish the stoichiometry for this type of system. Because of the multiplicity of binding sites per particle, and the vast range of aggregates that result from agglutination, conventional binding-theory is not applicable. It is for this reason that an empirical approach was used. This constitutes the first quantification of the binding of a lectin to a large particle, and was achieved *via* fluorimetric assay, using corn starch and concanavalin A that had been labelled with fluorescein isothiocyanate. The resulting data were arranged to yield an expression for the isotherm of binding, and a measure of the cooperativity.

EXPERIMENTAL

Materials and methods. — The corn starch used in this research was Kingsford's unmodified food-grade, made by CPC International*, Coventry, Connecticut. The labelled concanavalin A was purchased from Pierce Chemical Co., Rockford, Illinois, and from Miles Laboratories, Inc., Elkhart, Indiana; both batches were verified by sedimentation pattern, and measurement of the sedimentation constant, which showed them to consist of monodisperse tetramer. Tests for free fluorescein isothiocyanate in the preparations of concanavalin A were negative.

The fluorimeter was constructed in this laboratory. The sample cell was a standard, quartz, spectrophotometer cell of 1 cm² cross-section, with entrance and exit beams at a right angle. Wavelengths of the beams were set by Schoeffel monochrometers, model GM 250, bearing 2-mm slits, to 490 and 516 nm, respectively. The light source was a Hanovia xenon arc, model 901C0011. The detector was a 1P21 photomultiplier, and readout was *via* a Keithley electrometer, model 610B. A double, plano-convex condenser located between the xenon arc and the entrance monochrometer provided sufficient collimation to show a sharp slit image at the sample cell and at the photomultiplier. Electrometer readings ranged from 14 to 6000 mV. When necessary, a small correction, never larger than 6 mV, was applied to compensate for scattered light.

After calibrating the fluorimeter against known concentrations of concanavalin A, assay was conducted at room temperature. Aliquots of lectin ranging from 2.15 to 70 μ g were combined with amounts of corn starch ranging from 50 mg to 2 g, and the volume of each sample was made up to 5.00 mL with 0.01M Tris buffer, pH 7.84, which was 0.15M in NaCl, and 1mM in each of the salts CaCl₂, MgCl₂, and MnCl₂. After incubation for 30 min, the mixture was centrifuged to separate the lectin-starch complex from the unbound lectin. The latter was determined by observing the supernatant liquid in the fluorimeter. Then a 4% solution of methyl α -D-mannopyranoside (4 mL) was added as a competitor to the lectin-starch complex. After a 2-h incubation-period, this mixture was centrifuged, and the liberated lectin was determined in the fluorimeter. Material balance of the lectin indicated that there was a modicum of enhancement of the fluorescence, because the lectin accounted for exceeded the amount introduced into the system. Because the excess was never more than 6%, no correction for this was made. The validity of determining the concentration of lectins by fluorescence measurements was demonstrated by Monsigny *et al.*³, who showed that data obtained in this way are in agreement with data from radioassay.

All glassware used in this work was tested to be sure that it did not adsorb concanavalin A.

To make certain that the binding was reversible, portions of the lectin-starch

*Reference to brand or firm name does not constitute endorsement by the U.S. Department of Agriculture over others of a similar nature not mentioned.

complex were placed in contact with 0.01M Tris buffer, and, in all cases, the lectin was completely recovered. When the lectin was added to the starch during the assay procedure, agglutination occurred, and this was observed under a microscope. After the competitor was added to the starch-lectin complex, the starch returned to a monodisperse state.

RESULTS AND DISCUSSION

The results of the binding experiments are shown in the family of straight lines plotted in Fig. 1, as the extent of binding *versus* the mass of lectin introduced into the system over a moderate range of concentration of concanavalin A. Here, r is the ratio of bound concanavalin A (in μg), to the mass of starch (in mg), A_0 is the total mass of concanavalin A (in μg), and M_0 is the mass of starch (in mg). In each case, the extent of binding is linear, and shows a coefficient of linear correlation >0.999 . The slope of each plotted line increases as the mass of starch is lessened, which shows that decreasing the total number of binding sites exposed to a given mass of concanavalin A results in a relatively greater number of bound sites. At high values of M_0 , there is less concanavalin A bound per starch grain than at low values. Lower values of M_0 lead to increased binding per starch grain (at a given value of A_0), and thus, positive cooperativity prevails. This is substantiated by the Scatchard plot⁴ of one experiment, shown in Fig. 2, where A is the concentration of unbound concanavalin A (in μg per mL) at equilibrium. A Scatchard plot that shows a positive slope is indicative of positive cooperativity⁵. Scatchard plots were drawn for all of these experiments, and all were of the general shape of the curve in Fig. 2. Because the

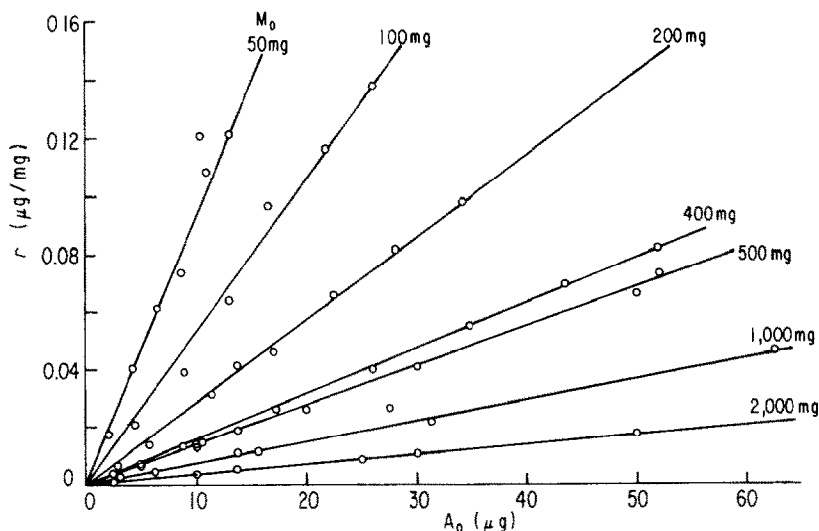


Fig. 1. Graph of extent of binding *versus* total lectin for the system concanavalin A-corn starch.

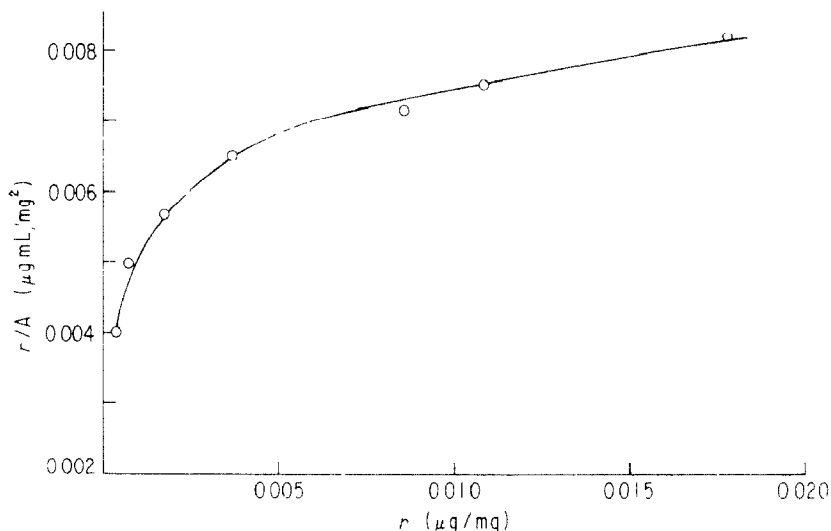


Fig. 2. Scatchard plot for the system concanavalin A-corn starch. [The mass of starch used was 2.000 g, and the mass of concanavalin A was varied from 1.25 to 50.0 μ g.]

concentration of concanavalin A used carried the Scatchard plots to almost zero slope, it appears that a state of saturation had been reached.

The conventional delineation of this type of data is a plot of r versus A (unbound), rather than r versus A_0 (total) as shown in Fig. 1. The latter approach was used, however, because it leads directly to the isotherm of binding.

In a typical application, parameters are obtained from the Scatchard equation. This is^{4,5}

$$R/A_u = kn - kR,$$

where R is the ratio of bound sites to total sites, A_u is the concentration of unbound lectin at equilibrium, k is a binding constant, and n is the total number of binding sites. The parameter k is a true equilibrium constant only in the case of noncooperative binding to identical sites; then, the Scatchard plot is a straight line of negative slope, with definite intercepts on both axes. Because of the shape of the curve in Fig. 2, it is not possible to obtain further numerical information. This aspect is further complicated by the fact that the system under study is an example of heterogeneous equilibrium, as both corn starch and the concanavalin A-starch complex are insoluble.

The straight lines that pass through the origin in Fig. 1 show the relation

$$r = eA_0, \quad (1)$$

where e is the slope of each plotted line. Plotting of these values of slope versus the corresponding mass of starch yielded a hyperbolic curve. When the same points

were plotted to logarithmic scales, the result was a straight line that followed the relation

$$e = gM_o^h, \quad (2)$$

with h equal to the slope of the line, and g , a constant. The method of least squares gives h a value of -0.88 , and the coefficient of linear correlation of the points plotted was calculated to be 0.999 , thus verifying the linearity of the line.

Substituting Eq. 2 in Eq. 1, and entering values obtained in the fluorimetric assays, gives the isotherm of binding of concanavalin A to corn starch:

$$r = 0.33 A_o M_o^{-0.88}. \quad (3)$$

This expression makes it possible to calculate the extent of binding, given the masses of starch (M_o) and concanavalin A (A_o) introduced into the system. It is also possible to calculate the mass, A_B , of bound lectin and the concentration, A , of unbound lectin from the relations

$$r = \frac{A_B}{M_o} = \frac{A_o - AV}{M_o}, \quad (4)$$

where V is the volume (in mL).

The coefficient g and exponent h are characteristic of the system. It is possible to consider g to be an equilibrium constant, as it holds for all values of the reactants. The index h is a measure of positive cooperativity, because it determines the slope

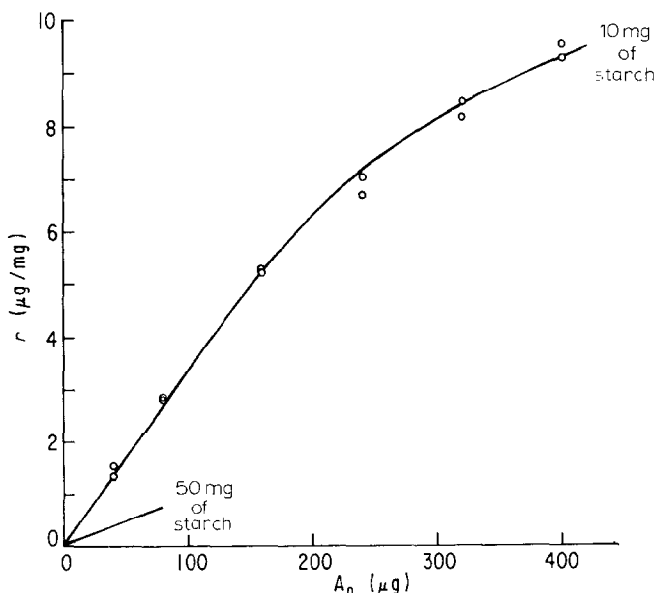


Fig. 3. Graph of extent of binding *versus* total lectin for the system concanavalin A-corn starch at high ratios of ligand to substrate.

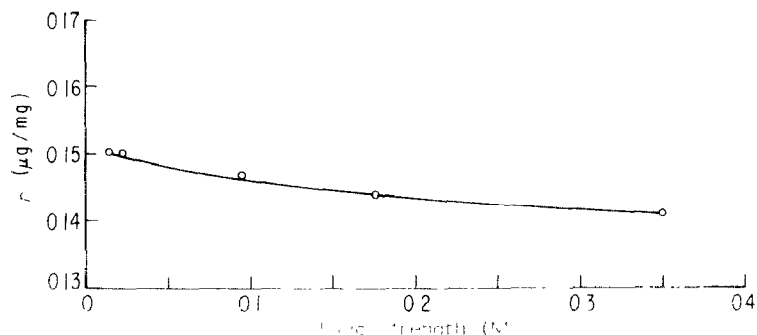


Fig. 4. Graph of the extent of binding *versus* ionic strength of the dispersion medium, for the system concanavalin A-corn starch.

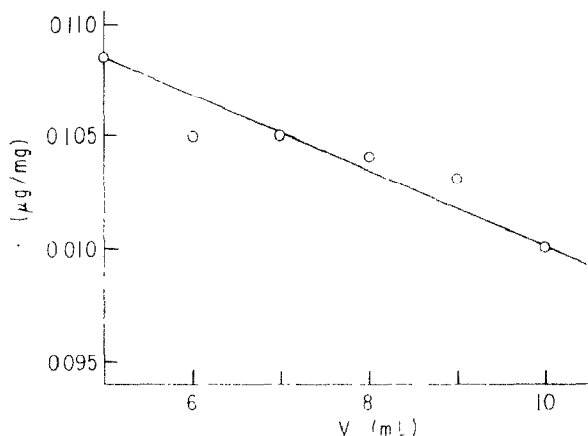


Fig. 5. Graph of the extent of binding *versus* volume for the system concanavalin A-corn starch. [Concanavalin A (70 μg) and 400 mg of starch were used.]

of the $\log e$ *versus* $\log M_0$ plotted line, and, the steeper the slope, the greater is the increase in bound sites relative to the decrease in mass of corn starch.

Extending these observations to very high ratios of concanavalin A to starch introduces saturation effects. The result of lowering the mass of starch to 10 mg, and increasing the mass of concanavalin A to 400 μg is shown in Fig. 3, where the extent of binding is plotted *versus* the total mass of the lectin; this curve shows a definite approach to a zero-slope asymptote beyond ~ 150 μg of lectin. The line for 500 mg of starch, shown in Fig. 1, is included here for comparison. Carrying these measurements into the range of milligrams of concanavalin A ultimately yields a downward curvature of the plotted line.

Varying the pH of the system over the range 7.54 to 8.04 produced no shift in values beyond the experimental error of 6%.

The influence of ionic strength on the binding of concanavalin A to corn starch is shown in Fig. 4, where the extent of the binding is plotted for 500 mg of starch and

100 μg of concanavalin A. In the range of ionic strength from 0.01 to 0.35, there is a 7% decrease in binding; this dependence tends to indicate that part of the binding is electrostatic. The small slope of the curve, however, suggests that this is but a small part of the binding force. Measurements were not carried to lower values of ionic strength, because of the difficulty in retaining the concanavalin A in solution under these conditions.

The effect of varying the volume of the system from the 5-mL value in the titrations reported is shown in Fig. 5, where the volume is extended to 10 mL while the ionic strength and pH are held constant, and the corresponding values of r are plotted for A_0 equal to 70 μg , and M_0 equal to 400 mg. The result is a straight line whose equation could be used to obtain a corrected value of r . In this case, doubling of the volume introduced an error of 9%.

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