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CONCANAVALIN A AGGLUTINABILITY OF DEXTRAN GEL SPHERES

A PHYSICAL MODEL FOR CELL AGGLUTINATION

PAUL Y. WANG [a], DONALD W. EVANS [a] and WALTER ZINGG [b]

[a] *Laboratory of Chemical Biology, Institute of Biomedical Engineering, Faculty of Medicine, University of Toronto, Toronto, Ontario M5S 1A4, and* [b] *the Research Institute, Hospital for Sick Children, Toronto, Ontario M5G 1X8 (Canada)*

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Summary

Dextran is a polysaccharide which can interact with concanavalin A, and insolubilized dextran spheres are available with different degrees of swelling in water. One grade of these dextran gel spheres can be agglutinated extensively by concanavalin A, while another grade with the same binding constant is not affected. Photomicrographs and measurement by capillary suction show that deformability and good contact between these gel spheres can be correlated with their agglutinability.

Introduction

Previous work in this laboratory deals with adhesion phenomena in the biological environment [1]. In the interaction of a synthetic polymer with internal tissue, it has been observed that when a reactive monofunctional compound consisting of six or seven carbon atoms is attached to the tissue surface, interfacial contact is facilitated and strong adhesion results.

Another system, which may provide some information on the complex problem of bio-adhesion, is suggested by our recent observation that concanavalin A can agglutinate one grade of cross-linked dextran gel spheres, whereas another grade binds the same amount of the lectin but is not agglutinated [2,3]. A similar difference in the concanavalin A agglutinability has been extensively studied for transformed and untransformed cells [4—6].

provide an explanation for the difference in agglutinability of the dextran gel spheres. The information obtained may be helpful to the study of cell agglutinability.

Materials and Methods

Concanavalin A

Twice-crystallized concanavalin A dissolved in saturated sodium chloride solution was obtained from Miles Laboratories, Elkhart, IN. Low molecular weight fragments were removed by gel filtration on Bio-Gel P-100 [8] and the existence of the protein essentially as the intact subunit was confirmed by polyacrylamide gel electrophoresis in sodium dodecyl sulfate. The concanavalin A preparation was checked for a full complement of metal ions (Ca^{2+} and Mn^{2+}) by its ability to bind to Sephadex [9]. Solutions were made up in phosphate-buffered saline, pH 7.4, and the concentration routinely determined by ultraviolet analysis (Unicam SP 1800 spectrophotometer) based on $E^{1\%}_{280}$ (1 cm path) = 13.0. The value of the extinction coefficient under these conditions was confirmed by dry weight analysis [7].

Dextran gel spheres

Cross-linked dextran gel spheres (Sephadex), grades G-75, G-100, and G-200 were obtained from Pharmacia, Montreal. Dry gel spheres of each grade were fractionated into narrow size ranges by sieving gently [10]. Fractions passing through a No. 120 sieve (125 μm), and retained on a No. 140 sieve (160 μl) were used. After hydration in phosphate-buffered saline, a well-shaken 1 : 1 (v/v) suspension of the gel spheres was further diluted 5-fold, and sampled with a 1 ml plastic serological pipet (Falcon, Oxnard, CA). The pipet had been preconditioned by drawing and discharging a gel sphere suspension several times, followed by rinsing three times with phosphate-buffered saline. The number and size distribution of the gel spheres in suspension were determined with a modified Neubauer hemocytometer [11] in which the depth of the counting chamber was increased from 0.1 to 0.3 mm to ensure unrestricted influx of the large gel patrticles. The number of spheres counted in the hemocytometer was subsequently converted to concentration (i.e., number of spheres per ml).

Agglutination assay

A volume of 1 ml of phosphate-buffered saline and a concanavalin A solution of 0.5 ml containing 0.2—10 mg/ml of the lectin were added to 35 × 10 mm style plastic Petri dishes (Falcon Plastic, Oxnard, CA). A volume of 0.2 ml of a well-shaken 1 : 1 (v/v) suspension of pre-swelled gel spheres in phosphate-buffered saline was then added to each dish by a 1 ml plastic pipet. The assay suspensions were then shaken horizontally at 20° C for 15 min on a metabolic shaker at 30 oscillations/min and an amplitude of 7 cm. Agglutination was scored visually: 0, no agglutination; +, slight; ++, low; +++, medium; ++++, extensive [12]. To study the effect of oyster glycogen on agglutination, a dilute solution of this polysaccharide (BDH Chemicals, Poole, U.K.) in phosphate-buffered saline (1 mg/ml) was introduced into the assay suspension by a microliter syringe (Hamilton Co., Whitter, CA).

Binding assay

The amount of concanavalin A bound to gel spheres at steady-state was determined by the change in protein concentration in solution [9]. In each binding experiment, a number of 60 × 15 mm style plastic Petri dishes containing 3.0 ml phosphate-buffered saline and 1.5 ml concanavalin A solution of a required concentration was prepared. A volume of 0.6 ml of a well-mixed 1 : 1 (v/v) suspension of dextran gel spheres was added by a 1 ml plastic pipet to each dish, and the covered dishes were maintained at 20° C while being shaken. For the control samples, methyl α-D-glucopyranoside was mixed at a final concentration of 0.1 M with the concanavalin A solution before the gel spheres were added. At various intervals the contents of an assay and that of a control dish were quickly centrifuged and the supernatant from each sample was withdrawn for ultraviolet spectrophotometric analysis at 280 nm (1 cm path). The binding experiments were continued until constant absorbance readings were obtained (4—5 h). The amount of concanavalin A remaining in solution at steady-state was calculated using the extinction coefficient, and the amount of the lectin specifically bound to the gel spheres was obtained by difference. The results obtained over a range of 300-fold concanavalin A concentration were plotted according to the method of Scatchard [13].

Deformability of gel spheres

The deformability of the dextran gel sphere was studied using the micropipet suction technique developed by Mitchison and Swann [14]. The experimental assembly was essentially as described previously by Weed and Lacelle [15]. Several gel spheres in phosphate-buffered saline were first placed in the modified hemocytometer to measure the dimeter under microscope. After filling the micropipet and the connecting tubing with phosphate-buffered saline, the tip of the capillary was brought up to a gel sphere and a slight negative pressure was applied to just sealing the sphere onto the opening of the pipet. Two sets of experiments were conducted to compare the deformability of different grades of gel spheres. In the first, the negative pressure required to just draw gel spheres of different diameters completely into a micropipet was determined using a manometer. In the second set, a series of readings of the linear deformation of a gel sphere with increasing negative pressure was obtained (Fig. 1). The deformation in μm was plotted against pressure in dynes/cm^2 to produce a curve, and the slope of which gave the 'stiffness' or resistance to deformation of the particle [14].

A simple compression test was also used to demonstrate the difference in deformability of the gel spheres in another way. A drop of a 1 : 1 (v/v) suspension of preswelled gel spheres in phosphate-buffered saline was placed on a microscope slide. As the drop gradually reduced in size due to evaporation, the liquid surface tension (~74 dynes/cm) gently compressed the spheres against each other. After allowing 20 min for contraction of drops comparable in size, photomicrographs were taken. The extent of contact between gel spheres under the standard compression condition was determined by measuring the projected length of contact between two surfaces and expressing it as a percentage of the sphere circumference.

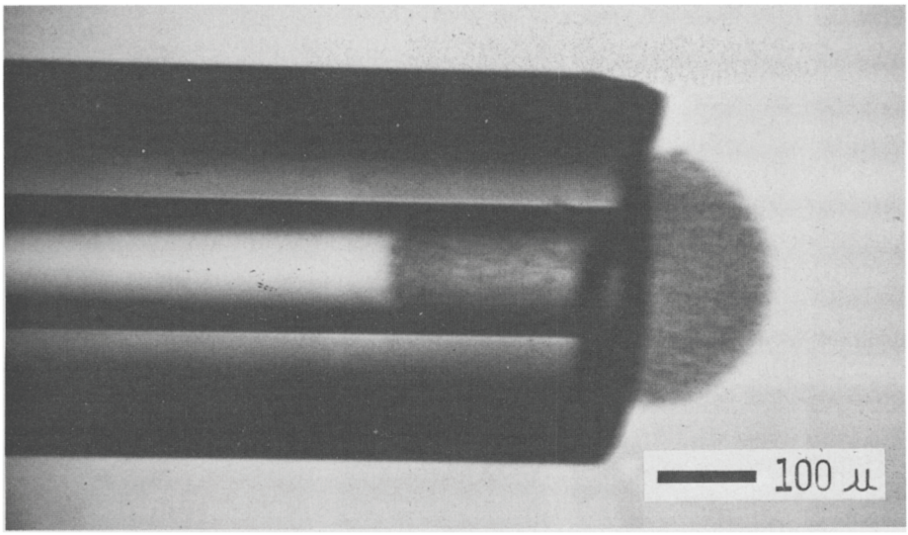

Fig. 1. A gel sphere is deformed under increasing negative pressure and advances into the micropipet. The internal diameter of the glass capillary is 130 μm.

Agglutination of gel discs

To observe the interfacial contact under agglutinating conditions and to facilitate focusing under microscope, dry dextran spheres were imbedded in paraffin and sliced into discs of 10 μm in thickness with a microtome. The paraffin was removed with hot water and the gel discs were transferred to phosphate-buffered saline. A small number of these discs were placed in concanavalin A solution (882 μg/ml) and shaken carefully to keep as many of them in the same horizontal plane as possible. Photomicrographs of the region of contact between surfaces were taken at a magnification of 400×.

Concanavalin A-glycogen interaction

The interaction was followed by measuring the absorbance of the dispersed molecular complex formed under various conditions. Samples containing 1.5 ml of a 882 μg/ml concanavalin A solution and 1.5 ml of freshly prepared oyster glycogen in phosphate-buffered saline (18—58 μg/ml) were mixed and absorbance monitored at 520 nm with a Unicam SP 1800 spectrophotometer. Reference samples contained, in addition, 0.1 M methyl α-D-glucopyranoside as inhibitor. The difference between the sample and reference readings was taken as the absorbance due to the concanavalin A-glycogen complex alone.

Results

After hydration, stock samples of the gel spheres showed a wide size distribution. The diameter of about 90% of the spheres was in the range of 50—250 μm for all grades. After fractionation by gentle sieving, over 90% of the spheres upon hydration had a diameter of 200 ± 20 μm. The 1 : 1 (v/v) suspensions of gel spheres, relatively uniform in size, contained $4.25 \cdot 10^5$, 4.67 ·

10^5, and $4.95 \cdot 10^5$ spheres/ml for grades Sephadex G-75, Sephadex G-100, and Sephadex G-200, respectively. After correcting for dilution due to addition of phosphate-buffered saline and the concanavalin A solution, the corresponding final concentrations were $5.00 \cdot 10^4$, $5.49 \cdot 10^4$, and $5.82 \cdot 10^4$ spheres/ml in the assay solutions.

At a concanavalin A concentration of 150 μg/ml, slight agglutination (+) of gel Sephadex G-200 was first observed. Maximum agglutination (++++) occurred at concentrations of 500 μg concanavalin A/ml and higher. Gel Sephadex G-100 was agglutinated by concanavalin A to a lesser extent (maximum: +++ at 900 μg/ml). At all concanavalin A concentrations studied, no agglutination was observed for gel Sephadex G-75. When a mixed sample of Sephadex G-200 and Sephadex G-75 spheres were assayed in the presence of concanavalin A, clumps of agglutinated spheres were observed in suspension consisting of Sephadex G-75 spheres surrounding individual or a cluster of Sephadex G-200 spheres (Fig. 2). The Sephadex G-75 spheres not associated with clumps remained dispersed. These mixed gel clumps were quite stable and showed no tendency to separate under the standard assay conditions.

Scatchard plots on the binding of concanavalin A to gels Sephadex G-200 and G-75 (Fig. 3) were essentially identical. A single class of non-interacting sites was observed in both cases with an association constant of about 10^5 M^{-1}. The number of binding sites/sphere, obtained by extrapolating the plots to $r/[A] = 0$ and multiplying the value at the intercept by $6.023 \cdot 10^{23}$ molecules/mol (assuming one molecule of concanavalin A occupies one site), was $2.59 \cdot 10^{11}$ for gel Sephadex G-200 and $2.77 \cdot 10^{11}$ for gel Sephadex G-75. When calculated based on the average hydrated sphere diameter of 200 μm, the results are $2.06 \cdot 10^{14}$ and $2.20 \cdot 10^{14}$ sites/cm^2, respectively. In control samples

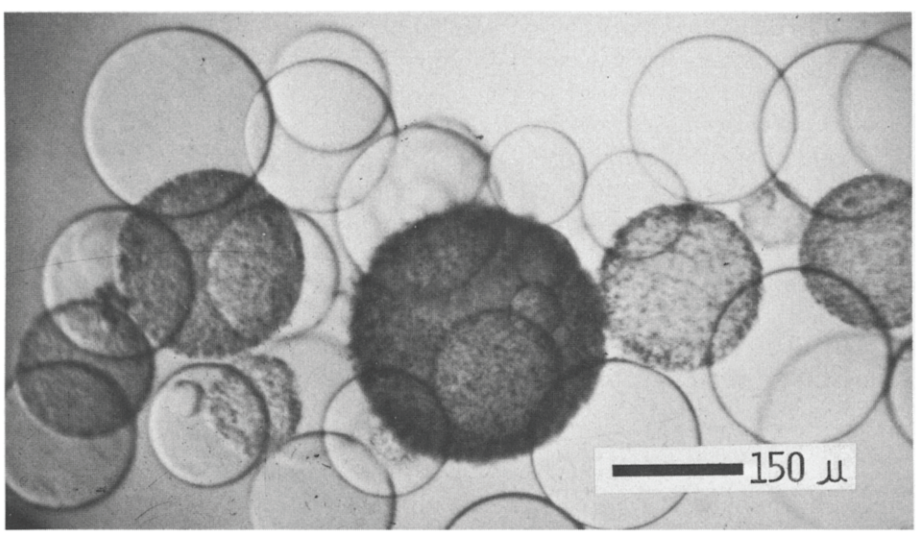

Fig. 2. Dextran gel spheres in concanavalin A solution. Unstained gel Sephadex G-75 spheres (light) agglutinated around stained gel Sephadex G-200 spheres (dark). The staining was effected by swelling the gel spheres in 0.1 M $AgNO_3$ solution, washing in 0.3% NaCl solution and resuspending in phosphate-buffered saline. This treatment had no effect on agglutination.

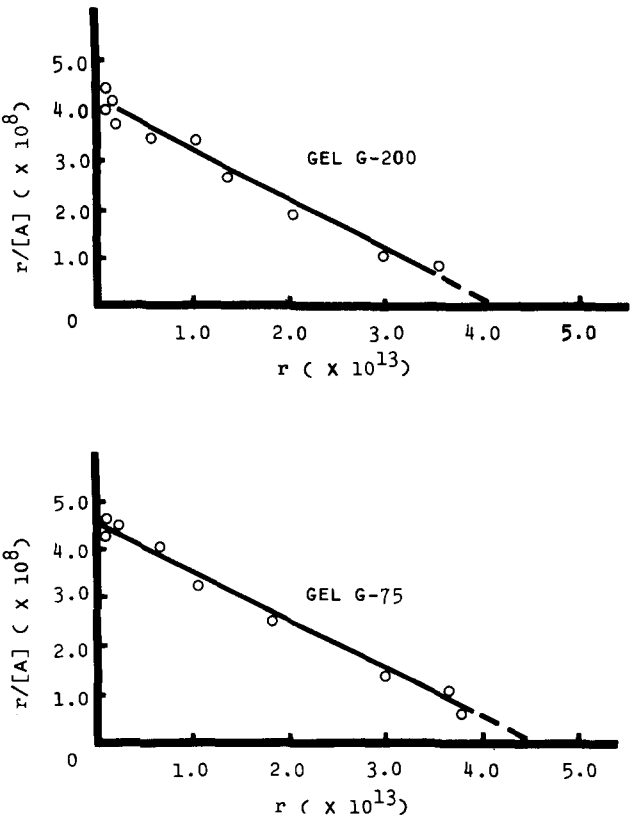

Fig. 3. Binding of concanavalin A to gel spheres according to Scatchard [10]. The change in concanavalin A concentration in solution due to specific binding on gel spheres was followed spectrophotometrically. The amount of concanavalin A bound in mol/sphere is represented by r, and [A] indicates the number of moles of free concanavalin A remaining in solution. The slope of each line in the graphs gives the association constant.

where the specific binding was inhibited by methyl-α-D-glucopyranoside, any change in the concentration of concanavalin A in solution would imply a penetration of the lectin into the interior of the gel spheres. However, no such concentration changes were detected for gel Sephadex G-75 or G-200. Therefore, under the experimental condition, the interaction of concanavalin A with the α-D-glucopyranosyl units of the dextran chain was limited primarily to the surface of the gel sphere.

The minimum pressure required to draw an individual sphere of gel Sephadex G-75, G-100 or G-200 completely into a 130 μm micropipet is shown in Fig. 4. This graph shows that the pressure observed for Sephadex G-75 spheres was about four times greater than that required for a Sephadex G-200 sphere of the same size. Similar results were obtained when the distance advance by the leading edge of a sphere along the micropipet was measured as a function of the negative pressure applied (Fig. 5). From the slope of the linear section of the upper curve shown in Fig. 5, the stiffness or resistance to deformation of gel Sephadex G-75 spheres was calculated to be 2.9 ± 0.3 dynes/cm per μm deformation. Values of 1.3 ± 0.2 and 0.7 ± 0.1 dynes/cm

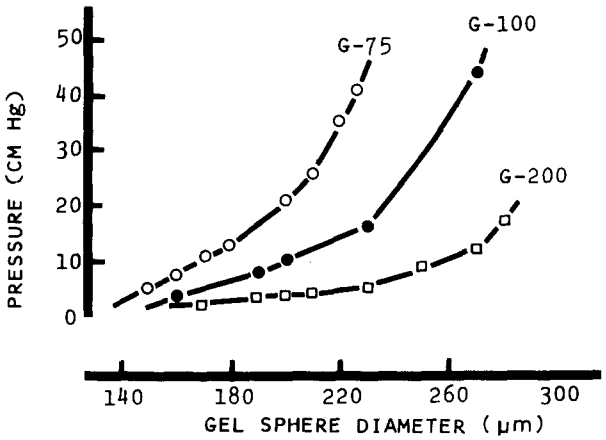

Fig. 4. Minimum pressure required to completely draw individual gel spheres of different diameters into a micropipet with internal diameter of 130 μm. Each point on the graph represents the average of at least ten separate measurements.

per μm deformation were obtained for gels Sephadex G-100 and G-200, respectively.

In the simple compression test, the surface of gel Sephadex G-200 was found to be very deformable with a large reduction in surface curvature at regions of contact with little distortion elsewhere. The projected lengths of contact between two spheres as viewed under microscope was about 15% of the circumference. By comparison, gel Sephadex G-75 spheres showed little more than point contact (Table I) under the same condition. When mixed samples were

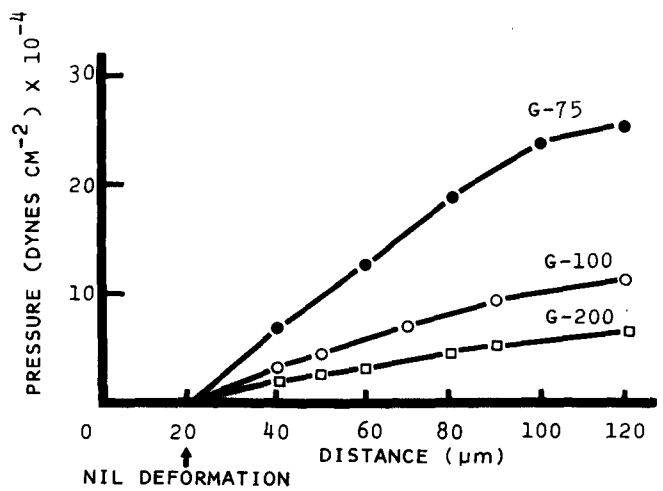

Fig. 5. Comparison of the resistance to deformation (stiffness) of gel spheres. A micropipet with internal diameter of 130 μm was used to assess the deformability of gel spheres through the distance advanced by the leading edge of the sphere inside the capillary (Fig. 1) under different increments of negative pressure. Ten spheres of each grade with diameters of 200 ± 20 μm were measured over the range of negative pressure indicated.

TABLE I

EXTENT OF CONTACT BETWEEN GEL SPHERES OR GEL DISCS

Each value represents the mean ± S.E. of ten determinations.

Gel	Contact as percentage of circumference	
	Sphere compression condition	Disc agglutination condition
G-75	3.8 ± 0.4	0
G-200	15.0 ± 2.1	6.5 ± 1.2
G-75/G-200	14.4 ± 3.0	6.7 ± 1.6

compressed, the relatively rigid gel Sephadex G-75 spheres were observed to indent the more deformable gel Sephadex G-200 surface (Fig. 6), and made similar extent of surface contact to that found between gel Sephadex G-200 spheres. The extent of surface contact under the agglutination condition as estimated from the gel disc experiments was about one-half that observed under the standard compression conditions (Table I).

Results from the study of the effect of glycogen on the agglutination of gel spheres are given in Table II. In the presence of 882 μg concanavalin A/ml, a small amount of oyster glycogen (10 μg/ml) did not affect the agglutination of gel Sephadex G-200. A slight increase in agglutination was observed for gel Sephadex G-100 when the same condition was applied. However, the addition of glycogen with concanavalin A produced extensive agglutination of gel Sephadex G-75, which was indistinguishable from that observed for gel Sephadex G-200 and concanavalin A alone. When gel Sephadex G-75 spheres were incubated with 882 μg concanavalin A/ml for 4 h (sufficient time for the

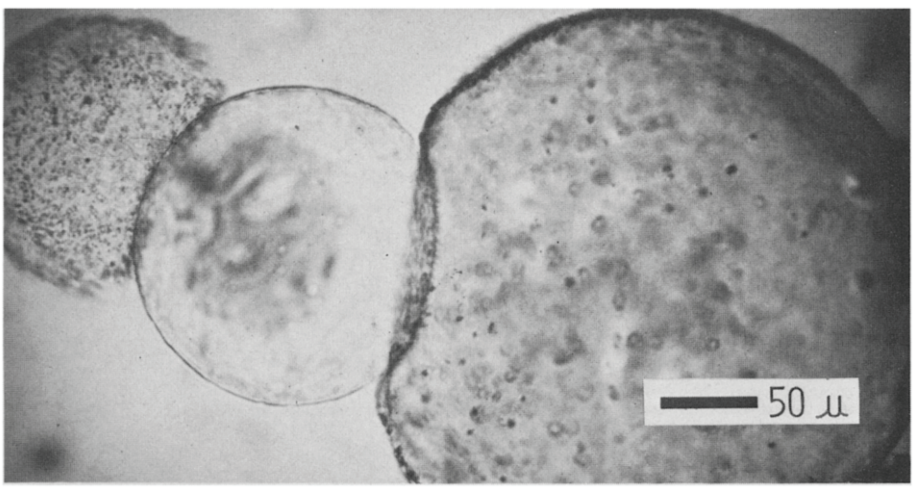

Fig. 6. Deformation of mixed gel spheres under compression. Drops of gel spheres suspension were allowed to evaporate on microscope slides. The evaporation reduced the size of the drops and compressed the gel spheres together by surface tension. Gel Sephadex G-200 spheres (dark) were stained and gel Sephadex G-75 (light) unstained. The staining was effected with AgCl as described in Fig. 2.

TABLE II

EFFECT OF GLYCOGEN ON AGGLUTINATION OF GEL SPHERES BY CONCANAVALIN A

The agglutination assays were done in triplicate.

Gel	Concanavalin A (μg/ml)	Glycogen (μg/ml)	Agglutination
G-200	882	0	++++
	882	10	++++
G-100	882	0	+++
	882	10	++++
G-75	882	0	0
	882	10	++++
	441	21	++++
	220	50	++++

binding to reach steady-state), washed thoroughly with phosphate-buffered saline and resuspended, the addition of glycogen alone (10 μg and up to more than 500 μg) did not result in agglutination. It appeared that the agglutination of gel Sephadex G-75 depended on a preformed concanavalin A-glycogen complex [16]. The formation of such a complex at different ratios of the two components was followed spectrophotometrically (Fig. 7). In the presence of 441 μg concanavalin A/ml, maximum agglutination of gel Sephadex G-75 spheres was obtained with the addition of 21 μg glycogen/ml. At lower glycogen concentrations, less complex formation was observed, and the extent of agglutination was reduced correspondingly (Fig. 7). With excess glycogen (greater than 21 μg/ml) the agglutination still occurred. However flocculent precipitates could now be seen in the assay suspension under microscope. Such insoluble concanavalin A-glycogen complexes usually adhered to the surface of the gel spheres and appeared between spheres in the agglutinated clumps [11].

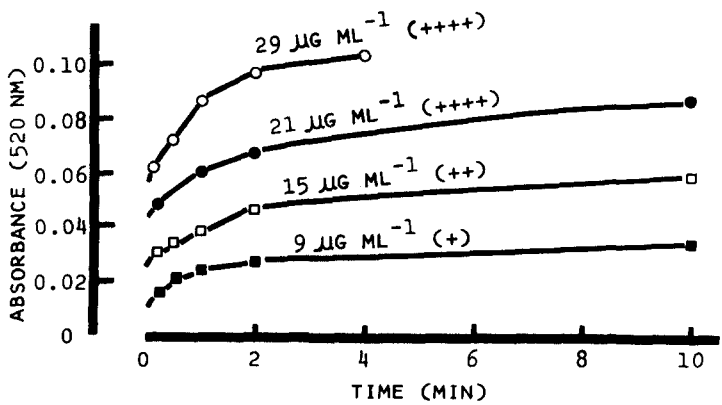

Fig. 7. Interaction of glycogen and concanavalin A. The complex formation was monitored spectrophotometrically. The solutions contained 441 μg concanavalin A/ml and different amounts of glycogen as indicated on each curve. The extent of agglutination of gel Sephadex G-75 produced by a specified condition is shown in the brackets on each curve.

Under all conditions, agglutinated gel spheres could be dispersed at any time by the addition of methyl-α-D-glucopyranoside (0.1 M), and glycogen (up to 1 mg/ml) in the absence of concanavalin A produced no agglutination even with gel Sephadex G-200 spheres.

Discussion

The dextran gel spheres are a relatively simple system with well-defined physical and chemical properties [17]. In a previous report [3], changes in the agglutination of gel Sephadex G-200 spheres due to molecular transition of the concanavalin A tetramer with temperature or pH have already been evaluated. In the present study, several relevant factors are examined to determine the most likely explanation for the difference in agglutinability observed for gel Sephadex G-75 and G-200.

The water swellability of the spheres and their penetrability by macromolecules when used in filtration chromatography depend on the degree of epichlorohydrin cross-linking of the dextran chains in the gel sphere. Gel Sephadex G-75 is impermeable to concanavalin A since the lectin has a molecular weight [18] of about 100 000. The exclusion limit of gel Sephadex G-200 is higher than G-75. However, simple calculation using the water regain value of gel Sephadex G-200 [11,17] can show that at a concentration of 882 μg concanavalin A/ml as used in the present study, only less than 6% of the lectin may be at the interior of the gel spheres if there is diffusion. In fact, no loss of concanavalin A from solution by such diffusion was detected under the assay condition in the presence of methyl-α-D-glucopyranoside. Therefore, it is highly unlikely that the vast difference in agglutinability observed can be attributed to the molecular exclusion limit of the gel spheres.

There are reports indicating that clustering of concanavalin A binding sites may be involved in determining agglutinability [4]. However, such clustering cannot occur on the gel surface, because the dextran chains are cross-linked by the epichlorohydrin reaction.

It is just as unlikely that the agglutinability is due to some aspects of concanavalin A binding. This conclusion is based on the results of Scatchard plots (Fig. 3). Gels Sephadex G-75 and G-200 were found to contain similar number of homogeneous, non-interacting bindings sites with essentially identical association constants.

However, there is a clear correlation between the stiffness or resistance to deformation of the gel spheres (Figs. 4 and 5) and the agglutinability. The deformability probably provides a better chance for mutual adaptation between the surfaces of gel spheres when they are being agglutinated in the presence of concanavalin A. On the other hand, the relatively rigid gel Sephadex G-75 spheres may not allow sufficiently close contact for the tetravalent concanavalin A molecules to form cross-bridges that will hold the spheres together. This inference is supported by the observation that in mixed gel samples, the rigid gel Sephadex G-75 spheres can agglutinate with gel Sephadex G-200 spheres (Fig. 2). The deformability of gel Sephadex G-200 spheres with respect to the more rigid and non-agglutinable gel Sephadex G-75 spheres can be demonstrated in the simple compression test (Fig. 6).

Additional support was obtained from experiments designed to adjust for insufficient contact between surfaces. The adjustment, if accomplished, is known to improve adhesion [1,19]. For this purpose, the agglutinating agent was expanded in size by interacting concanavalin A with glycogen to form a complex [16]. The multivalent glycogen is a homopolysaccharide composed of α-(1 → 4)-D-glucopyranosyl units with α-(1 → 6)-linked branch chains. In the presence of excess concanavalin A, binding sites on glycogen forming the complex may be bound to the tetravalent concanavalin A still having valencies available for further interaction. The concanavalin A-glycogen complex thus formed should function better than the concanavalin A molecule alone in overcoming insufficient contact between gel Sephadex G-75 spheres. The addition of a small amount of glycogen together with concanavalin A in solution indeed resulted in extensive agglutination of gel Sephadex G-75 spheres (Table II). Fig. 7 indicates that the extent of agglutination increases with increasing concentration of the complex.

The free energy change in the binding of concanavalin A to dextran gel spheres is about −7 kcal/K at 20°C as readily calculated from the association constant. This value is comparable to the interactions between antigen and antibody [20]. The immunoglobulin molecule has flexible regions to accomodate the varied molecular topography of determinant sites on an antigen. However, concanavalin A does not appear to possess the same molecular feature. Therefore, surfaces that are sufficiently flexible to allow a close mutual adaptation should conceivably have a better chance of being bridged by concanavalin A resulting in agglutination as in the case of gel Sephadex G-200.

In conclusion, our observation from the study of a physical model for concanavalin A induced agglutination is in agreement with data from recent experiments using red blood cells [6].

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References

1 Wang, P.Y. (1974) Nature 249, 367—368
2 Wang, P.Y. and Evans, D.W. (1977) Fed. Proc. 36 (3), 795
3 Evans, D.W. and Wang, P.Y. (1978) in Carbohydrate-Protein Interaction (Goldstein, I.J., ed.), pp. 76—89, ACS Symposium Series, Washington, DC
4 Sharon, N. (1977) Sci. Am. 236, 108—120
5 Marquardt, M.D. and Gordon, J.A. (1975) Exp. Cell Res. 91, 310—316
6 Gordon, J.A. and Kuettner, C.A. (1978) Nature 272, 636—638
7 Agrawal, B.B.L. and Goldstein, I.J. (1967) Biochim. Biophys. Acta 147, 262—270
8 McKenzie, G.H. and Sawyer, W.H. (1973) J. Biol. Chem. 248, 549—556
9 Uchida, T. and Matsumoto, T. (1972) Biochim. Biophys. Acta 257, 230—234
10 ASTM E-11, Annual Book of ASTM Standards (1976) Part 14, Am. Soc. Test. Mater., Philadelphia
11 Evans, D.W. (1978) Ph.D. Thesis, University of Toronto
12 Sela, B., Lis, H., Sharon, N. and Sachs, L. (1970) J. Membrane Biol. 3, 267—279
13 Scatchard, G. (1949) Ann. N.Y. Acad. Sci. 51, 660—672

14 Mitchison, J.M. and Swann, M.M. (1954) J. Exp. Biol. 31, 443—461
15 Weed, R.I. and Lacelle, P.L. (1969) in Red Cell Membrane Structure and Function (Jamieson, C.A. and Greenwalt, T.J., eds.), pp. 318—388, Lippincott, Toronto
16 Goldstein, I.J., Reichert, C.M., and Misaki, A. (1974) Ann. N.Y. Acad. Sci. 234, 283—293
17 Sephadex Gel Filtration in Theory and Practice (1974) pp. 4—9, Pharmacia Canada, Dorval, Quebec
18 McKenzie, G.H., Sawyer, W.H. and Nichol, L.W. (1972) Biochim. Biophys. Acta 263, 283—293
19 Baier, R.E., Shafrin, E.G. and Zisman, W.A. (1968) Science 162, 1360—1368
20 Kabat, E.A. (1977) Structural Concepts in Immunology and Immunochemistry, p. 143, Holt, Reinehart and Winston, New York