The Role of Concanavalin A Dissociation on Positive Cooperativity of Binding with Native and Fixed Erythrocytes*

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Positive cooperativity demonstrated by Scatchard plot analysis of concanavalin A (con A) binding was found with native or glutaraldehyde-fixed erythrocytes. This suggests that factors other than membrane changes might be involved in the apparent increase in receptor binding affinity with increasing site occupancy.

The elution pattern of 125I-con A chromatographed on Bio-Gel P-150 with decreasing concentration showed a drop in average molecular weight compatible with con A dissociation to dimers, protomers, and protomer fragments. Similarly, the per cent of 125I-con A specifically binding to Sephadex G-75 fell with decreasing concentration of con A applied. The inclusion of unlabeled carrier con A suppressed the dissociation of labeled con A in Bio-Gel P-150 and increased the per cent binding to Sephadex G-75.

Both labeled and unlabeld con A were multibanded in sodium dodecyl sulfate-polyacrylamide gel electrophoresis. As previously reported, the three major bands are consistent with intact protomer (~25,000 daltons) and two fragments (~13,000 and 10,000 daltons) with minor bands representing undissociated species.

These observations indicate that there is a concentration-dependent association of Con A subunits which contribute to the observed positive cooperativity of con A binding to erythrocytes.

Of considerable biological interest is the study of the structure and function of cell membranes. The interaction of ligands such as hormones with specific receptors on the surface of cells can initiate a series of membrane and intracellular events altering the growth and behavior of cells (1-3). If the ligand is a component of another cell surface or acts as a bridge between the cell membranes, the initial event can take the guise of specific cell recognition, adhesion, or aggregation (4, 5).

A model of these biological phenomena and important in its own right is the interaction of lectins with cell(s); lectins can initiate intracellular events, identify surface components, act as bridges between cells, and mimic the action of insulin (6-8). Membrane components also have been identified which act as lectins (9, 10).

A detailed understanding of ligand-protein and ligand-receptor interactions in solution or on surfaces begins with a quantitative measurement of the ligand to receptor interaction. The biological consequences of such a reaction follow this initial interaction (1, 3, 11).

The interpretation of binding by the familiar Langmuir isotherm and Scatchard plot analysis (12) appears to be easily made (13, 14) and allows for meaningful analysis with reservations (15-17). In addition to the usual parameters which can be derived from a Scatchard plot, a distinction between association reactions which are mediated by the ligand or facilitated by the interaction of the ligand can be made by varying the protein or cell concentration (17, 18). Such a study was in mind when we sought a closer analysis of the interaction of lectins with cell surfaces by means of binding experiments. Before the effect of cell concentration on lectin binding could be determined, a closer analysis of the binding interaction at low lectin concentration was required.

Recently published binding data for concanavalin A (19-21), wheat germ agglutinin (22), and soybean agglutinin (23) to cell surfaces when analyzed by Scatchard plots manifest distinct positive cooperativity at low concentrations of lectin; the explanation for the increasing affinity of the lectin with increasing concentration has been based on some form of ligand-induced membrane alteration (4, 22, 23). We wondered whether an explanation of positive cooperativity of binding might lie with the polymeric form of native con A. We report that con A dissociates to protomer and protomer fragments at very low concentration; the associating behavior of con A occurring with increasing concentration contributes to the positive cooperativity of binding to cells. The observed positive cooperativity of con A binding to fixed erythrocytes would apparently diminish the role of the cell membrane changes in this behavior.

EXPERIMENTAL PROCEDURES

Preparation of Erythrocytes—Outdated blood bank blood was washed in phosphate-buffered saline and the upper 5% of the cell mass discarded with each of the three successive washes. A final wash was done in PBS containing 1 mM CaCl₂ and 1 mM MgCl₂. The cells were then suspended in PBS-CM as a 1 x 10⁹ cells/ml stock (approximately 2% v/v) as determined on a Bio/Physics cytograph, model 6300. Cells were used within 2 h of preparation.

Washed erythrocytes fixed with 2.5% glutaraldehyde as a 10% suspension overnight were extensively washed and stored at 4°C. Prior to use, three additional washes in PBS and finally PBS-CM gave a cell suspension of 1 x 10⁹ cells/ml (approximately 2% v/v).

Iodination of Concanavalin A—Crystalline con A (Miles-Yeda) was labeled by the chloramine-T method (24). As we detected no effect of adding mannose in the concanavalin A solution to be iodinated, it was not included in the preparations described below. To 20 mg of con A in 1 ml of PBS-CM was added 150 µl of dimethyl sulfoxide (Malinckrodt) and the solution was cooled on ice (25). Two milliliters of carrier-free 125I (New England Nuclear, NEZ-033L) in 20 µl of PBS was added and immediately followed by 200 µl of chloramine-T.

The abbreviations used are: con A, concanavalin A; PBS, phosphate-buffered saline; PBS-CM, PBS plus 1 mM CaCl₂ and MgCl₂; αMM, α-methylmannoside; SDS, sodium dodecyl sulfate.

* This work was supported by National Institutes of Health Grant CA-14213 to J. A. G. Department support is provided by National Cancer Institute Grant CA-15823. The costs of publication of this article were defrayed in part by the payment of page charges. This article must therefore be hereby marked "advertisement" in accordance with 18 U.S.C. Section 1734 solely to indicate this fact.
Con A Dissociation and Binding

(1 mg/ml, Eastman Organics) in PBS-CM. After 5 min the reaction was terminated with 200 μl of potassium metabisulfite (2.4 mM) and the mixture applied to a Sephadex G-100 column (0.9 x 25 cm) at 4°C. The column was washed with PBS-CM until the eluted radioactivity reached a uniform low level (<10^6 cpm/ml) and then eluted with mannose (10 mg/ml) in PBS-CM. The central fractions of the single peak were pooled, dialyzed until negligible radioactivity passed the dialysis bag, and stored as aliquots at -20°C. The specific radioactivity of the two preparations of radioactive con A were 23.4 and 24.7 x 10^6 cpm/μg of protein.

The labeled con A was assayed for protein by the method of Lowry (26), titrated for activity with trypsinized human erythrocytes (27), and assayed for unbound radiodiode. The labeled con A preparations showed more than 95% of the label remaining with the lectin when assayed by thin layer chromatography (28).

Polyacrylamide Gel Electrophoresis—Polyacrylamide gel electrophoresis on slabs was carried out after the method of Laemmli (29) with the following modifications: the samples (20 μg/ml to 2 mg/ml) were mixed with an equal volume of sample buffer which was 2% in SDS, 10% sucrose, 0.14 M EDTA, 80 mM dithiothreitol, and pyronin Y marker. Following 30 min incubation at 60°C, the samples (5 to 15 μl) were applied to a 10-cm-long gradient gel (7.5% to 15%) through a 4% stacking gel and electrophoresed at 10 V/cm. The gels were fixed and stained in 25% isopropyl alcohol, and 10% acetic acid solution containing 0.04% Coomassie blue (30).

Binding of Con A to Erythrocytes at Room Temperature—A stock solution of 1.0 mg/ml con A (1 v/v) at one of several concentrations was allowed to incubate with Sephadex G-75 (31) contained in a small column (0.7 x 7 cm) for 30 min and then "unbound" con A washed out with 16 ml of PBS-CM, at which time the final 1 ml fraction contained little radioactivity. A sharp peak of "bound" radioactivity (Table I) was then eluted with 100 mM α-methylmannopyranoside. The sum of the recovered unbound and bound radioactivity equaled the total known radioactivity applied to the column within experimental error (<1%). The elution fractions were individually counted on a Beckman Bio-Gamma counter.

In some cases, unlabeled con A was added as "carrier" (1 mg/ml final concentration) to the labeled con A solution. In addition, cell-free supernatants from labeled con A solutions previously incubated with erythrocytes (10% v/v) in PBS-CM for 2 h at room temperature were also analyzed for their con A binding behavior to the Sephadex G-75 column.

Binding of Iodinated Con A to Erythrocytes at Room Temperature—Erythrocytes (1 x 10^8 cells/ml) were mixed with an equal volume of various concentrations of labeled con A diluted from frozen stock and prior to use. The total suspension was gently agitated for 2 to 2.5 h when duplicate 100- to 150-μl aliquots were removed to establish the total radioactivity and cell binding. Additional aliquots were removed at 5 h. The binding of labeled con A to cells was assayed by a modified Microfuge technique (32). Briefly, the cell suspension was layered over 1 ml of 5% solution of bovine serum albumin in a 1.5-ml Microfuge tube (Beckman Instruments). The erythrocytes were obtained by centrifugation was washed with 1.5 ml of PBS-CM and counted in the Microfuge tube. The free (unbound) con A was taken as the radioactivity in the bovine serum cushion and cell pellet wash. Recovery of radioactivity ranged between 96 and 100%; binding of labeled con A to the Microfuge tube in the absence of the cells ranged between 0.5 to 2% of the radioactivity which was determined to be cell-bound.

Inhibition and reversal studies were carried out in 100 mM oMMP added before con A addition or following 2 h incubation, respectively. Comparison was made to cell suspensions without added inhibitor. Labeled con A was assayed for its ability to compete for binding sites on washed erythrocytes in the presence of unlabeled con A. A at a level of total con A giving approximately 50% saturation of binding sites (25 μg of con A/ml) the labeled preparation was fully competitive with unlabeled con A present at 5, 25, 50, and 75% of the total.

RESULTS

Properties of 125I-Labeled Con A—Con A (labeled with sodium 125I) was purified as a single peak by affinity chromatography with a specific activity of 23.4 and 24.7 x 10^6 cpm/μg obtained for the two con A preparations used. Labeled con A showed no loss or change of biological structure or function by any of several criteria. Labeled and unlabeled con A agglutinated normal and trypsinized erythrocytes following 2-fold dilutions down to concentration levels of 1.5 to 2.0 μg/ml of protein when determined by Microfuge plate analyses (27).

Binding of Con A to Native and Fixed Erythrocytes—Binding to washed erythrocytes at room temperature was carried out by a slight modification of the Microfuge technique described by Phillips et al. (32). In the absence of cells, no significant radioactivity penetrated the bovine serum albumin cushion, or remained with the Beckman microcentrifuge tube.

| Con A | Exposed* | Unexposed* |
|-------|----------|------------|
| µg/ml |          |            |
| 0.16 (0.19) | 57 | 49 | 11 |
| 0.5 | 49 | 49 |
| 1.0 | 43 | 40 | 11 |
| 1.3 (2.5) | 47 | 22 |
| 2.0 | 13 | 12 | 11 |
| 2.5 (5.4) | 12 |
| 10 | 11 |
| 47 (63) | 11 |
| 100 | 11 |
| 307 (304) | 11 |

* The number enclosed by parentheses represents the original con A concentration exposed to washed erythrocytes. The concentration of con A remaining in the supernatant was derived from the counts per min remaining divided by the specific activity of labeled con A.

1000 μg of unlabeled con A (as 40 mg/ml) added to 1 ml of unexposed labeled con A which was assayed for binding to Sephadex as outlined above.

Con A Dissociation and Binding

One milliliter of con A in PBS was applied to a 3-ml column of Sephadex G-75 (0.9 cm diameter) at room temperature and allowed to incubate for 30 min after the initial run-in. The per cent unbound material represents the per cent of total 125I-con A applied to the affinity column which eluted with the first 16 ml of PBS divided by the total con A (counts per min) applied. Elution with α-methylmannoside plus the unbound con A gave a total recovery between 96 to 101%.

Table I

Concentration dependence of 125I-con A binding to Sephadex G-75 with and without prior exposure to cells

| Per cent con A unbound | Carrier | No Carrier |
|------------------------|--------|-----------|
| 0.5 | 49 | 11 |
| 1.0 | 49 |
| 1.3 (2.5) | 40 | 11 |
| 2.0 | 22 |
| 2.5 (5.4) | 12 |
| 10 | 11 |
| 47 (63) | 11 |
| 100 | 11 |
| 307 (304) | 11 |
Con A Dissociation and Binding

FIG. 1. Densitometer tracing of SDS-polyacrylamide gel electrophoresis patterns of native and 125I-con A purified by affinity chromatography. The 7.5 to 15% gradient gels were stained with Coomassie blue, dried, and scanned with a Joyce-Loebl densitometer in the reflectance mode. Approximate molecular weights were established through the use of protein markers in adjacent cells. The 125I-con A differed from native con A as indicated by the dotted lines.

Eliminating the step requiring the removal of the upper portion of the tube prior to analysis. Nevertheless, bookkeeping of all fractions was maintained with total recovery of the radioactive counts generally ranging between 98 to 102%. The counts remaining with the upper layer and albumin cushion and cell wash were taken as the "unbound" concanavalin A while the counts with the cell pellet represented "bound" concanavalin A. In general, agreement between duplicate or triplicate aliquots were within 1%. As a further control, we found that 98 to 99.8% of the originally bound radioactivity remained with the cells following resuspension and washing in PBS CM.

Note that the binding experiments, including the binding assays, were all carried out at room temperature (22 ± 1°C) to avoid the temperature-dependent depolymerization of concanavalin A (27). In contrast to other reports (19), we are not confident that full equilibrium was reached in less than 2 h (Fig. 3A). In our experiments, the cells were typically assayed at 2.5 and 5 h of incubation without any differences noted. The specificity of concanavalin A binding was demonstrated by the release of radioactivity with α-methylmannopyranoside added 2 h after incubation (Fig. 3B). Inhibition studies with 100 mM αMM reduced the level of con A binding to 2 to 3% of normal. No correction is therefore necessary or required for the nonspecific interaction of lectin.

The experimental results of con A binding to native and fixed erythrocytes are treated by a Langmuir isotherm (Fig. 4). For clarity, a single curve joins all the points although small significant differences at low lectin concentrations are seen (Fig. 4, inset). The results of three other experiments were identical or even showed slightly greater coincidence of

FIG. 2. The competition of labeled with unlabeled con A shown as the micrograms of labeled con A bound to erythrocytes. Incubation was carried out for 3 h with 1% native (○) or 1% trypsinized (△) erythrocytes (v/v) at room temperature in a total of 20 μg/ml of labeled plus unlabeled con A, and assayed for binding as described under "Experimental Procedures."

FIG. 3A, the extent of con A bound per cell shown as a function of time. Saturation at 3.6 × 10−13 μg/cell reached after 1.5 h. Ten percent native erythrocytes (v/v) incubated with 50 μg/ml of 125I-con A at room temperature. B, labeled con A remaining bound after 2 h incubation with 125I-con A, then reversed by various concentrations of αMM. Ten percent erythrocytes incubated with 50 μg/ml of 125I-con A at room temperature. The presence (+) or absence (−) of cell agglutination was determined by Microtiter plate assay after reversal.

FIG. 4. Langmuir isotherm analysis of con A bound to 1% native (●) and fixed (○) erythrocytes after a 3-h incubation at room temperature. The binding of con A at low concentrations to erythrocytes is shown by the inset.
and 1% fixed (○) erythrocytes and 1% native (●) and 1% fixed (○) erythrocytes after 3 h incubation at room temperature. Experimental data identical with that used in Fig. 3 plotted according to the method of Scatchard (12). Because the molecular species of con A binding to erythrocytes is questionable, “bound” and “free” con A on the ordinate is expressed as micrograms/ml rather than as molar quantities. See “Results.”

The determination of molecular sizes at several concentrations of labeled lectin was next examined. Utilizing a Bio-Gel P-150 column, concentrations of 10 or 25 μg/ml of labeled con A was applied in 1-ml aliquots. Resolution of molecular weights of above 67,000 was not fully satisfactory on our columns (either 0.9 × 20 cm or 0.9 × 40 cm). However, the use of larger columns would have diluted the radioactivity to unacceptably low levels for analysis. Nevertheless, the following points are quite clear (Fig. 6).

1. A relatively greater per cent of label is found in the area between the peaks with decreasing labeled lectin concentration.

2. A large family of molecular species first exits the gel at volume fractions dependent on the initial concentration of labeled lectin applied.

3. A relatively greater per cent of label is found in the area between the peaks with decreasing labeled lectin concentration.

4. A more uniform family of larger molecules is obtained with all concentrations of labeled con A in the presence of unlabeled carrier con A.

Recovery of the total radioactivity applied to the columns was 96% and 90 to 80%, respectively, in the presence of absence of carrier through elution of 5 void volumes. Radioactivity binding behavior of unexposed solutions of con A with changes in concentration suggested that exposure of cells did not remove a more active fraction of con A from the supernatants or lead to degradation after 3 h incubation. When we added unlabeled carrier con A (1 mg/ml), uniform binding of the labeled con A was seen at all concentrations applied. For example, at 2 μg/ml of con A applied, 60% of the labeled lectin bound in the absence of carrier and more than 85% in the presence of carrier con A. The 11% reported as unbound in the presence of carrier represents a small peak of material eluting with the first wash plus that leaking off with extensive washing before elution with 0.5M. The distribution of molecular species found in these preparations of con A were examined by SDS-gel electrophoresis, the distribution obtained from unlabeled con A, labeled con A, and labeled con A remaining in the supernatant following exposure to cells changed neither in their distribution nor their relative band intensities (identical with Fig. 1).

As positive cooperativity was seen when the binding of labeled con A to fixed erythrocytes was examined (Fig. 5), the role of membrane receptor mobility seemed an unlikely explanation for this behavior. Examining the behavior of the con A remaining in the supernatants exposed to cells, we found that the per cent of labeled con A binding to Sephadex G-75 (with a capacity of 15 mg of con A) significantly increased with increasing concentration of con A (Table I). The similar results between normal and fixed cells at lower concentrations of lectin. The data describing the interaction of con A with normal and fixed erythrocytes is also displayed by means of the Scatchard method of analysis (12):

\[
B/F = -K_B + K_n n
\]

where \( B \) is the micrograms of con A bound per ml, of suspension, \( F \) is the concentration of free lectin expressed as micrograms per ml. The association constant, \( K_n \), then is the negative value of the slope, and the value of \( n \), the maximum amount of con A bound per cell, is the intercept on the abscissa. As the distribution of molecular species of con A is unknown, representation as micrograms of lectin bound per ml, of suspension for this behavior. Examining the behavior of the con A remaining in the supernatants exposed to cells, we found that the per cent of labeled con A binding to Sephadex G-75 (with a capacity of 15 mg of con A) significantly increased with increasing concentration of con A (Table I).
from the Bio-Gel P-150 columns continued to leak at very low levels especially in the absence of carrier con A. As the addition of aMM (not shown) did not seem to change this behavior, it would appear that there is some nonspecific interaction of con A with Bio-Gel P-150.

DISCUSSION

We have shown that the per cent of labeled con A specifically binding to Sephadex G-75 in PBS-CM falls with decreasing concentration. At high concentration, more than 89% of unlabelled con A and labeled con A binds to Sephadex G-75 in PBS-CM (Table I). The addition of unlabelled con A (1 mg/ml) to low concentrations of labelled con A raises the per cent labeled con A bound to equal that of labeled con A at high concentration. We show that labeled con A competes for erythrocyte receptors with unlabelled con A on a 1:1 basis (Fig. 2). In addition, the biological behavior of the labeled preparation was identical with unlabelled con A when examined for its agglutinating behavior directed toward trypsinized erythrocytes (minimum effective concentration of 1.5 μg/ml and aMM demonstrated specific inhibition). We, therefore, conclude that our iodination of con A followed by affinity column purification altered neither the biological nor the binding behavior of con A.

Analysis of labeled and unlabelled con A by SDS-gel electrophoresis on 7.5 to 15% polyacrylamide gels demonstrated five or so bands when stained by Coomassie blue. This result is in agreement with previous reports which suggest a molecular distribution consisting of protomers, two major protomer fragments, and some associated species under denaturing conditions (30, 35). Under nondenaturing conditions, we found that chromatography on Bio-Gel P-150 eluted with PBS-CM clearly demonstrated a family of molecular weights dependent on the concentration of con A (Fig. 6). Much of the con A at lower concentrations eluted with larger V/V₀ ratios which suggested it was composed of smaller molecular weight polypeptides. These results are consistent with a shift of molecular weight from tetramers (or oligomers) to dimers, protomers, and smaller fragments with decreasing con A concentration. Dissociation into the smaller molecular weight species was effectively suppressed by the addition of carrier con A (Fig. 6). From these results, including the concentration-dependent binding to Sephadex G-75, we conclude that the dissociation of labeled and unlabelled con A to lower molecular weight species (which do not bind or bind weakly to acceptors) is concentration-dependent. By comparison with the results obtained under denaturing conditions, we suggest that tetrameric con A undergoes significant dissociation into dimers, protomers, and protomer fragments at lectin concentrations below 5 μg/ml, with an overall loss in binding affinities.

When the binding isotherm of a ligand rises steeper than the curve for statistical binding, the apparent affinity of the receptor is usually thought to increase as the ligand occupancy of sites increases (11, 36). On a Scatchard plot, this is expressed as a curvilinear shape which is bell-shaped, i.e. the curve is concave downward at lower concentrations of ligand (11). Our results (Fig. 5) on the binding of con A to erythrocytes demonstrate positive cooperativity when plotted according to the method of Scatchard (12). These results are similar to those reported by others for con A (19) and soybean agglutinin (29). Failure to find cooperative binding of lectins to membrane surfaces might be more a limitation of experimental design, i.e. lower concentration ranges were not examined, or improperly drawing linear graphs (37).

The detailed explanation of Scatchard plots demonstrating positive or negative cooperativities is not always clear. Deviations in the expected statistical binding in simpler systems are usually based on mechanisms involving conformational changes in the protein induced by ligand binding (Ref. 38 and references therein). Nichols and his co-workers (15-17) have shown that polymerization reactions involving the ligand, the protein, or both, can give rise to nonstatistical binding showing positive cooperativity. Even with systems simpler than that examined here (i.e. the binding of organic dyes with albumin in solution or small molecules to membranes (39)), there is much controversy regarding the mechanistic significance derived from the apparent positive or negative cooperativity. Recently, elegant mathematical modeling of these situations have been explored in detail (15-17).

Positive cooperativity of ligand binding to membrane surfaces introduces other explanations which generally involve changes in receptor mobility, or the membrane itself, or both (4). For example, the positive cooperativity in the Scatchard plot seen with the binding of chlorpromazine to erythrocytes was explained on membrane or cell shape changes (39). From the shape of the Langmuir isotherm and the enhancement of labeled con A binding by additional unlabelled con A, Cuatrecasas (11) proposed a cooperative interaction of membrane receptors on isolated adipocytes as an explanation. Similarly, Reisner suggests that the cooperative binding of soybean agglutinin as a positive manner to normal and trypsinized erythrocytes at low concentration may result from 1) cross-linking of receptors by lectins, 2) unmasking of cryptic sites, or 3) conformational changes of a nonspecific nature in the membrane. Other recent reports on the binding of lectins to Chinese hamster ovary cells (22) cells derived from a teratoma (21) and thymocytes (20) similarly invoked a mechanistic explanation based upon alterations in the membrane, receptor, or receptor mobility.

Although much attention has been given to membrane, or receptor influences on binding behavior, or both, the effects on the binding curves resulting from changes in the lectin or other ligand have not been studied. Yet many examples are now known where isomerization or self-interaction of small ligands, such as dyes or substrates binding to proteins, cause deviations of the binding curve from a simple hyperbolic function. On both theoretical and numerical grounds, Nichols and his colleagues have convincingly demonstrated that ligand self-interaction may operate to accentuate or obscure cooperative binding to noninteracting as well as self-interacting acceptors, such as polymeric proteins (15-17). Therefore it is clear, in those cases, that binding curves suggestive of positive cooperativity of binding can stem from ligand associations rather than acceptor self-interactions. In the concentration region where the binding of con A shows positive cooperativity of binding to erythrocytes, we find that the lectin is associating into molecular species with greater binding affinities to Sephadex G-75. Not only does con A bind to native erythrocytes with positive cooperativity, but it also binds in a cooperative fashion to fixed erythrocytes where receptor mobility or membrane function changes are unlikely.

In conclusion, we note that the binding of lectins or other ligands to cell surfaces can be a complex phenomenon. Although membrane alterations represented as an equilibrium between monomeric and polymeric receptors have been widely recognized as influencing binding behavior, polymeric or isomerization changes of the ligand have been generally overlooked. A full understanding of the binding behavior (3) is dependent on factors influencing the relative binding affinities of the monomer or polymeric forms of the ligand to the cell. The overall experimentally determined association constant, K, for receptor binding of the ligand is then dependent on ligand-ligand and receptor-receptor as well as simple ligand-receptor reactions. Nonbinding species must be also
evaluated, whether these represent polymers, monomers, or ligand fragments. Conceivably, the association of ligands, such as epidermal growth factors (40), other hormones (3, 8, 11), or extracellular factors leading to cell-to-cell recognition provides additional regulation parameters of cell behavior.

REFERENCES
1. Birnbaumer, L., Pohl, S. L., and Kaumann, A. J. (1974) Adv. Cyclic Nucleotide Res. 4, 239-281
2. Rodbard, D. (1973) Adv. Exp. Med. Biol. 36, 342-364
3. Triggle, D. J. (1978) in Receptors in Pharmacology (Smithies, J. R., and Bradley, R. J., eds) Vol. 11, pp. 1-66, Marcel Dekker, Inc., New York
4. Singer, S. J. (1976) in Surface Membrane Receptors (Bradshaw, R. A., Frazier, W. A., Merrell, R. C., Gottlieb, D. I., and Hogue-Angeletti, R. A., eds) pp. 1-24, Plenum Press, New York
5. Bell, G. L. (1978) Science 200, 618-627
6. Lis, H., and Sharon, N. (1977) in The Antigens (Sela, M., ed) Vol. IV, Academic Press, New York
7. Cuatrecasas, P., and Tell, G. P. E. (1973) Proc. Natl. Acad. Sci. U. S. A. 70, 485-489
8. Goldfine, I. D. (1970) in Receptors in Pharmacology (Smithies, J. R., and Bradley, R. J., eds) Vol. 11, pp. 335-377, Marcel Dekker, Inc., New York
9. Stockert, R. J., Morell, A. G., and Scheinberg, I. H. (1974) Science 184, 365-368
10. Den, H., and Malinzak, D. A. (1977) J. Biol. Chem. 252, 5444-5448
11. Cuatrecasas, P., and Hollenberg, M. D. (1976) Adv. Protein Chem. 30, 251-451
12. Scatchard, G. (1949) Ann. N. Y. Acad. Sci. 51, 660-672
13. Weder, H. G., Schildknecht, J., Laiz, R. A., and Kesselring, P. (1974) Eur. J. Biochem. 42, 475
14. Janin, J. (1973) Prog. Biophys. Mol. Biol. 27, 94-97
15. Nichol, L. W., Jackson, W. J. H., and Winzor, D. J. (1967) Biochemistry 6, 2449-2456
16. Nichol, L. W., Smith, G. D., and Ogston, A. G. (1969) Biochim. Biophys. Acta 184, 1-10
17. Nichol, L. W., and Winzor, D. J. (1976) Biochemistry 15, 3015-3019
18. Cann, J. R. (1978) Methods Enzymol. XLVIIIF, 289-307
19. Schnebli, H. P., Lustig, A., Zulauf, M., Winterbitaler, K. H., and Joss, U. (1977) Exp. Cell Res. 105, 151-157
20. Bornens, M., Karsenti, E., and Avrameas, S. (1976) Eur. J. Cell Biol. 65, 61-69
21. Gachelin, G., Buc-Caron, M.-H., Lis, H., and Sharon, N. (1976) Biochim. Biophys. Acta 436, 825-832
22. Stanley, P., and Carver, J. P. (1977) Proc. Natl. Acad. Sci. U. S. A. 74, 9059-9069
23. Reisner, Y., Lis, H., and Sharon, N. (1976) Exp. Cell Res. 97, 445-448
24. Greenwood, F. C., Hunter, W. M., and Glover, J. J. (1963) Biochem. J. 89, 114-123
25. Stagg, B. H., Temperley, J. M., and Rochman, H. (1970) Nature 228, 58-59
26. Lowry, O. H., Rosebrough, N. J., Farr, A. L., and Randall, R. J. (1951) J. Biol. Chem. 193, 265-275
27. Gordon, J. A., and Marquardt, M. D. (1974) Biochim. Biophys. Acta 332, 136-144
28. Rosenberg, A., and Teare, F. W. (1976) Anal. Biochem. 77, 289-292
29. Laemmli, U. K. (1970) Nature 227, 680-685
30. Fairbanks, G., Steck, T. L., and Wallach, D. F. H. (1971) Biochemistry 10, 2606-2617
31. Goldstein, D. J. (1972) Methods Carbohydr. Chem. 6, 114-127
32. Phillips, P. G., Furmanski, P., and Lubin, M. (1974) Exp Cell Res. 86, 301-308
33. Abe, Y., Iwabuchi, M., and Ishii, S.-I. (1971) Biochem. Biophys. Res. Commun. 45, 1271-1278
34. Cunningham, B. A., Wang, J. L., Pflumm, M. N., and Edelman, G. M. (1972) Biochemistry 11, 3233-3239
35. Wang, J. L., Cunningham, B. A., Blauer, G. M., and Edelman, G. M. (1975) J. Biol. Chem. 250, 1490-1502
36. De Meyts, P., Bianco, A. R., and Roth, J. (1976) J. Biol. Chem. 251, 1877-1888
37. Vlodavsky, I., and Sachs, L. (1975) Exp. Cell Res. 93, 111-119
38. Colosimo, A., Brunori, M., and Wyman, J. (1975) in Protein-Ligand Interactions (Sund, H., and Blauer, G., eds) pp. 3-14, Walter de Gruyter, Berlin
39. Lovrien, R., Tisel, W., and Pesheck, P. (1975) J. Biol. Chem. 250, 3136-3141
40. Weber, G. (1975) in Protein-Ligand Interactions (Sund, H., and Blauer, G., eds) pp. 15-26, Walter de Gruyter, Berlin
41. Young, M., Salde, J. D., Murphy, R. A., and Blanchard, M. H. (1978) Biochemistry 17, 1490-1498
The role of concanavalin A dissociation on positive cooperativity of binding with native and fixed erythrocytes.

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J. Biol. Chem. 1979, 254:1932-1937.

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