ALTERED COOPERATIVE EFFECTS IN THE BINDING OF CONCANAVALIN A TO A VARIANT MAMMALIAN CELL LINE

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1. Introduction

Concanavalin A (con A), a plant lectin, is known to cause a variety of interesting but complex biological effects upon cultured mammalian cells; these diverse effects are probably initiated through the actual binding of the lectin to specific oligosaccharide chains at the surface membrane [1,2]. Some recent studies on con A binding have shown that the lectin interacts with the cell surface of some mammalian cells in a cooperative fashion. For example the lectin, but not its succinyl-derivative, binds to rat thymocytes in a cooperative manner [3]; local interaction of con A with mouse B lymphocyte receptors alters in a cooperative way the behaviour of cellular structures that are involved in the mobility of various cellular receptors and in the movement of the cell [4]; also cooperativity in the binding of con A to membrane-bound enzymes has recently been described [5,6].

It is apparent that a proper understanding of the mechanism involved in the binding of con A at the cell surface is important because it may lead to a clearer understanding of some basic principles of membrane function. It is likely that studies with mammalian variants exhibiting altered con A binding properties would provide additional insight into the problem. We have described selection procedures for obtaining lectin-resistant cells [7,8] and have recently described in detail the complex phenotype of a con A-resistant hamster cell-line [9]. In this report we show that con A binds to wild-type cells in a cooperative fashion and that this mechanism of con A binding is altered in the variant cells.

2. Materials and methods

Chinese hamster ovary (CHO) cells were grown at 34°C in α-minimal essential medium (Flow Laboratories, Inc.) supplemented with antibiotics and 10% (v/v) fetal bovine serum (Reheis Chemical Co.) as previously described [7]. The selection procedure [8] and the properties [9] of the con A-resistant variant have been described in detail.

In preparation for con A binding studies, wild-type and con A-resistant cells were grown on 15 × 60 mm tissue culture plates to about 1.8×10^6 cells/plate. In order to reduce the possibility of endocytosis and nonspecific binding the plates were incubated at 0°C for 5 min prior to the addition of labelled con A [10]. The cells were then washed with cold 0.154 M sodium chloride solution and incubated at 4°C for 5 min in phosphate-buffered saline (PBS) containing the appropriate concentrations of ^{3}H-labelled con A (New England Nuclear) in final vol. 2.0 ml. Preliminary experiments indicated that maximum binding occurred within the 5 min period. The cells were then washed five times with cold 0.154 M sodium chloride solution and solubilized in 10% Triton X-100 (J. T. Baker Chemical Co.) for 60 min at 37°C. The digested samples were added to an aquasol cocktail (New England Nuclear) and counted in a liquid scintillation counter. There was specificity in the binding of labelled con A since the amount of binding at all concentrations tested in the presence of 0.2 M methyl α-D-mannoside was 15% or less of the binding which normally occurred in the absence of the inhibitor. The quantity of binding in the presence

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of the sugar was considered to be nonspecific and was routinely monitored and substracted from the binding data obtained in the absence of the hapten.

3. Results and discussion

The binding of labelled con A to wild-type CHO cells as a function of lectin concentration is shown in fig.1. Apparently the binding curve obtained was not hyperbolic and suggested that con A was binding to the intact cells with positive cooperativity. When the data in fig.1 was analyzed for possible cooperative effects by means of a Hill plot [11] it became obvious that a significant amount of lectin was bound to the cells in a cooperative manner (fig.1, see inset); the Hill coefficient was calculated to be 1.8. However, when the binding of labelled con A to the con A-resistant cells was examined as a function of lectin concentration (fig.2) the binding curve appeared to be hyperbolic. When the data from fig.2 was analyzed by a Hill plot, the calculated Hill coefficient was 1.0 indicating a lack of cooperativity in the binding of labelled con A to the variant cells (fig.2, see inset).

The data in figs 1 and 2 were also analyzed by Scatchard plots [12]; the data is shown in fig.3. Clearly, con A bound to wild-type cells in a cooperative fashion, as judged by the non-linearity of the Scatchard plot. Conversely a linear Scatchard plot, which indicates a lack of cooperativity, was obtained

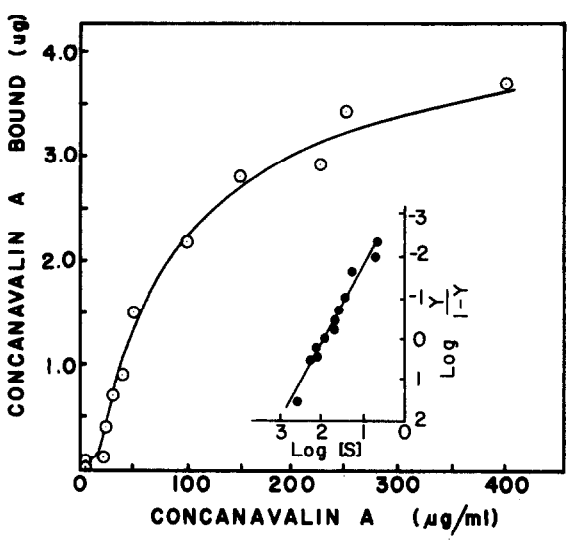

Fig.1. ^{3}H-Labelled con A bound per 1.8 × 10^{6} wild-type cells at various concentrations of lectin. Inset: data presented in the form of a Hill plot [11]. Hill coefficient was calculated to be 1.8.

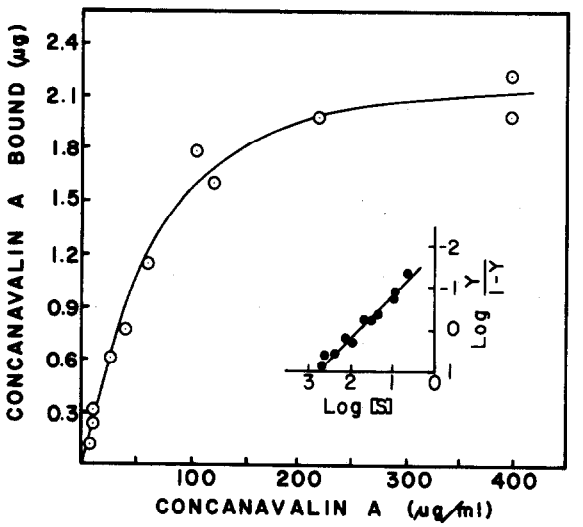

Fig.2. ^{3}H-Labelled con A bound per 1.8 × 10^{6} variant cells at various concentrations of lectin. Inset: data presented in the form of a Hill plot [11].

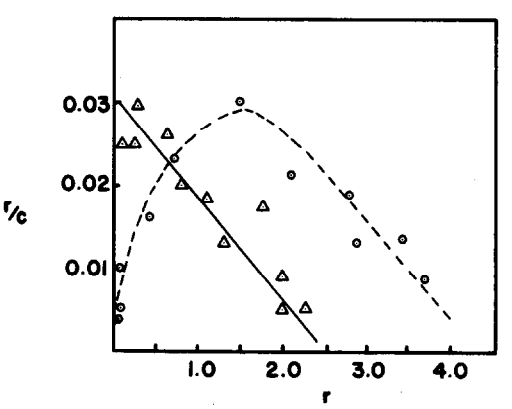

Fig.3. Data from fig.1 (○, wild-type cells) and fig.2 (△, variant cells) plotted according to Scatchard's equation [12]: $r/c = nK - rK$ where, r represents the amount of lectin bound, c is free lectin concentration, n is the amount of lectin bound at saturation and, K is the apparent association constant for lectin–receptor site binding. Each point represents the average of duplicate points from a single experiment; similar data has been obtained in 3 separate experiments.

when the lectin-resistant cells were incubated in the presence of labelled con A.

The cooperative binding observed with wild-type cells was not due to the presence of an equilibrium mixture of dimer and tetramer species of con A, which are probably present at pH 6.0 and above [1], since the binding of lectin to the variant cell-surface did not appear to involve cooperative effects. Although we do not know the precise mechanism involved in the binding of con A to wild-type cells it is probable that the binding of the first lectin molecules to the cell surface alters the surface in such a way as to expose new lectin receptor sites. A similar mechanism has been suggested for the binding of lectin to thymocytes [2] and for the interaction of human growth hormone with the human erythrocyte membrane [13].

The lack of cooperativity in con A binding with the variant cells may be due to changes in cell surface structures which prevent or modify the ability of the cell membrane to undergo the conformational changes observed with wild-type cells. The binding data (fig.3) suggests that the variant cells are only capable of binding between 55% and 65% as much lectin/cell as compared to wild-type cells; this suggests that either the variant cell-surface contains carbohydrate modifications or some potential receptor sites normally exposed in wild-type cells are present, but due to an altered cell-surface [9], they are not available for con A binding in variant cells. Also, recent preliminary studies with another independently isolated con A-resistant line has shown that these cells exhibit similar binding characteristics to the variant cells described in this report.

Therefore, we have been able to show that CHO cells bind con A with positive cooperativity; that mammalian variants with altered con A binding properties can be obtained in cell culture; and have described the altered lectin binding properties of one of these variant cell lines. Also, the properties of the variant cells suggest new approaches for investigating the cooperative interaction of multivalent ligands with cell-surface receptors. For example, lectin-resistant cells would be useful tools in studies attempting to link cooperativity in lectin binding with the fluidity properties of the surface membrane and the presence or absence of modified cell-surface structures.

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