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Note

Perturbation of the calcium-binding site in concanavalin A by a saccharide ligand

RONALD J. DOYLE AND STEPHEN D. STROUPE

Department of Microbiology and Immunology and Department of Biochemistry, University of Louisville Schools of Medicine and Dentistry, Louisville, Kentucky 40232 (U. S. A.)

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In recent years, lectins have become indispensable tools in the study of mitogenesis, mammalian cell-surfaces, and such immunologic phenomena as delayed hypersensitivity (reviewed by Liener[1]). One of the lectins, concanavalin A (con A), has received considerable attention because of its specificity of interaction with sugar-containing molecules and its ease of purification. In attempts to explain the diversity of the biological properties of con A, its sequence[2] and tertiary and quaternary structures[3–5] have been determined. In order that con A may exhibit its full, saccharide-binding potential, two metal-sites must first be occupied[6,7]. The first site, S1, can be filled with either Co^{2+}, Mn^{2+}, Ni^{2+}, or Zn^{2+}. The binding of transition metal results in the creation of a Ca^{2+}-specific site (S2), which, when filled, activates the saccharide-binding site. The two metal-sites are located ~500 pm apart in the con A molecule[3,5].

Considerable controversy surrounds the location of the carbohydrate-binding site[4,8,9] on con A. The data of Hardman and Ainsworth[4] and Becker *et al.*[9] suggest that the carbohydrate-specific site is on the surface of the lectin and close to the Ca^{2+} site. Hardman and Ainsworth predicted that saccharide would provide additional stability to the metal-binding regions. However, as pointed out by these authors, Ca^{2+} coordinates with some of the same side-chains thought to be involved in the carbohydrate-binding site. In this communication, we show that the affinity of con A for Ca^{2+} is lessened in the presence of methyl α-D-mannopyranoside (Meα-DMan*p*), a saccharide that binds to the lectin. The results suggest that Ca^{2+} and saccharide compete for the same binding-sites on the con A molecule.

EXPERIMENTAL

Con A was prepared by affinity chromatography according to Agrawal and Goldstein[10]. Metal-free con A was prepared as described previously[11]. A subunit molecular weight of 25,600 was used for calculations. Con A concentrations were

determined by absorbance measurements[12] at 280 nm, assuming that $E_{1\%,\,1\,cm} = 11.4$. All con A solutions were 5mM in Mn^{2+}.

Equilibrium dialysis was conducted with 100μM solutions of the lectin in 0.3M sodium chloride, 0.1M acetate (pH 5.3) for 41 h at 4°. The concentrations of Ca^{2+} inside and outside the dialysis bags were determined by scintillation counting of ^{45}Ca. The Scatchard equation, $r/c = K_a(n-r)$, was used to obtain K_a, by plotting r/c *versus* r, where r is the molar ratio of bound Ca^{2+} to the protein, and c is the molar concentration of free calcium. The slopes were determined by a least-squares method. Methyl α-D-mannopyranoside was purchased from Sigma Chemical Co., St. Louis, Missouri.

RESULTS AND DISCUSSION

Fig. 1 shows an equilibrium-dialysis experiment in which the association constant for the Ca^{2+}–con A complex was determined in the absence and presence of mM Meα-DMan*p*. In the absence of this saccharide, the K_a for Ca^{2+} binding was found to be 1.3×10^4 M^{-1}, in close agreement with the results of Shoham *et al.*[7]. However, when the experiment was performed in the presence of Meα-DMan*p*, the association constant was lowered to 7.1×10^3 M^{-1} and was not accompanied by a lessening in the

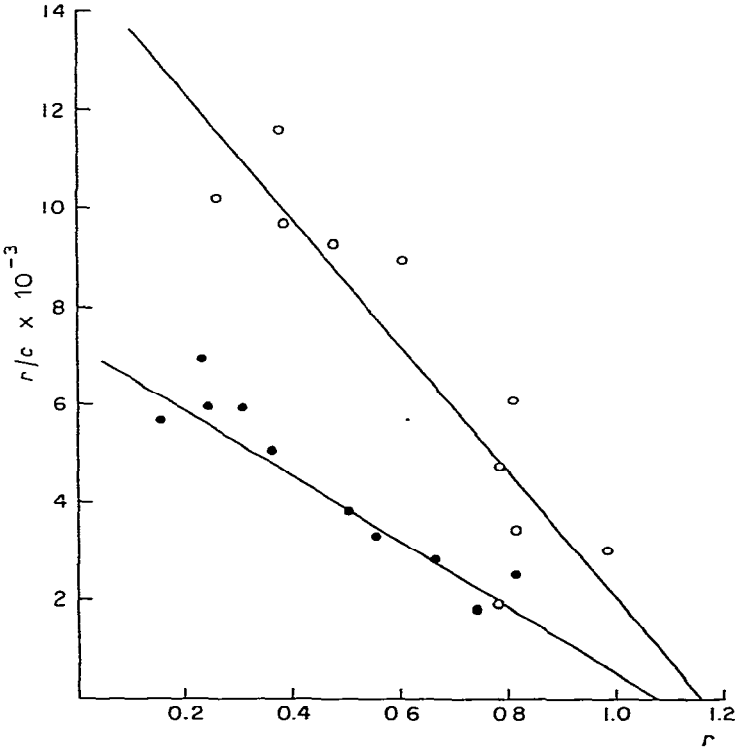

Fig. 1. Binding of Ca^{2+} to concanavalin A in the presence and absence of saccharide. [$^{45}Ca^{2+}$, —○—; $^{45}Ca^{2+}$ plus mM Meα-DMan*p*, —●—.]

number of sites. In both cases, approximately one mole of metal was bound per mole of lectin.

The chemical modification of tyrosine side-chains by con A leads to a diminished interaction with polysaccharide[13]. Hardman and Ainsworth[4] found that both Tyr-12, and Asp-208 are involved in binding of Ca^{2+} and saccharide. Becker *et al.*[9] suggested that additional ligands may be common to the Ca^{2+} and carbohydrate sites. The last two reports and the data shown in Fig. 1 suggest that saccharide and Ca^{2+} may actually compete for ligands. Accordingly, additional equilibrium-dialysis experiments were performed, using a constant concentration of Ca^{2+} and varied proportions of Meα-DMan*p*.

Fig. 2 offers a more direct confirmation that Ca^{2+} and Meα-DMan*p* compete when binding to con A. Con A was incubated with a constant amount of $^{45}Ca^{2+}$ and the samples were dialyzed at 4° against solutions containing increasing concentrations of Meα-DMan*p*. The ratio of mol of bound $^{45}Ca^{2+}$ per mol of con A, *r*, is plotted in Fig. 2 as a function of the log of concentration of Meα-DMan*p*. The solid curve is a theoretical curve obtained by a least-squares analysis of the data linearized in the

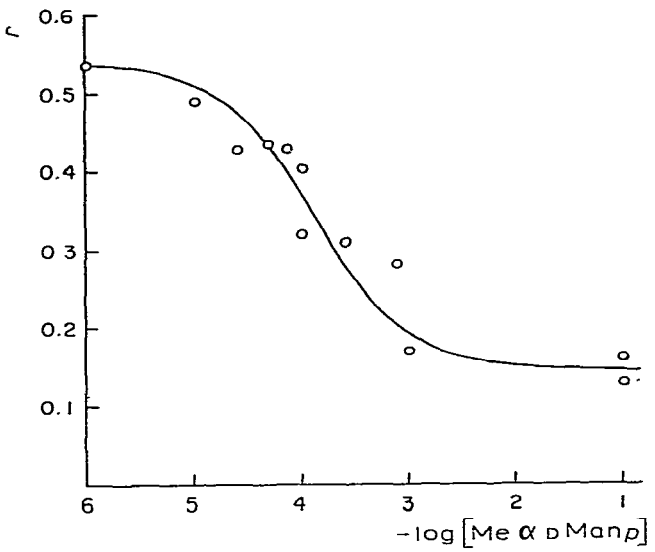

Fig. 2. Inhibition of Ca^{2+}–concanavalin A binding by methyl α-D-mannopyranoside. [Equilibrium dialysis was conducted as described. The concentrations of concanavalin A and Ca^{2+} were 59.5μM and 100μM, respectively.]

form of the Hill equation. The inhibition of Ca^{2+} binding was non-cooperative, and gave a K_a for binding of Meα-DMan*p* to con A of 7.3×10^3 M^{-1}, in reasonable agreement with literature values[14]. In Fig. 1, it was seen the Meα-DMan*p* competes with binding of Ca^{2+} by lowering the K_a for Ca^{2+}, not by destroying binding-sites. Therefore, from the four-fold lowering in *r* at saturating (0.1M) Meα-DMan*p* seen in Fig. 2, the K_a for binding of Ca^{2+} to con A is lessened four-fold in the presence of

high levels of Meα-DMan*p*. We suggest that, when Meα-DMan*p* interacts at the carbohydrate-binding site, the amino acid residues involved in binding of Ca^{2+} may shift their positions, resulting in a lessened affinity for Ca^{2+}.

Brewer *et al.*[15] and Brown *et al.*[16] suggested that the Ca^{2+} ion is not an absolute requirement for the activation of the saccharide-specific site in con A. Rather, they considered that Ca^{2+} enhances the rate of binding of Mn^{2+} to the protein. Richardson and Behnke[17] offered direct support for the premise that Ca^{2+} is not necessary for carbohydrate binding. We have been unable to show, using difference spectroscopy[11], that Meα-DMan*p* complexes with con A in the absence of Ca^{2+}. Furthermore, Mn^{2+} generates[11] an ultraviolet difference-spectrum in apo-con A, and immediately retards the thermal denaturation of the protein[18]. Thus, the exact role of Ca^{2+} in the structure and function of con A remains obscure. Additional refinements in the crystal structure of con A in the presence and absence of metal and saccharide ligands will be necessary in order that the interrelationships between the various binding-sites may be characterized.

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