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A CONFORMATIONAL CHANGE IN CONCANAVALIN A DETECTED BY A CALORIMETRIC STUDY OF THE BINDING OF METHYL α-D-MANNOPYRANOSIDE

G. R. Munske, J. A. Magnuson and H. Krakauer

Program in Biochemistry and Biophysics
Washington State University
Pullman, WA 99164

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SUMMARY

The binding of methyl α-D-mannopyranoside to the lectin concanavalin A was studied by means of calorimetry. An apparent enthalpy of binding was also calculated from the variation of the equilibrium constant with temperature (van't Hoff ΔH). The ΔH measured directly was -30 to -38 kJ/mole indicating that the binding is driven by the ΔH change. In contrast, the van't Hoff ΔH was substantially smaller, about zero at pH 5.2. The difference in the ΔH measured directly and the van't Hoff ΔH implies that the conformation of concanavalin A undergoes a temperature dependent change at both pH's but most predominantly at pH 5.2. The existence of this conformational change was verified by difference absorption spectroscopy.

Concanavalin A (Con A) is a lectin readily obtainable in pure form from the jack bean. It possesses sites to which sugars, most notably α-D-mannopyranoside, can bind specifically (1,2). Associated with this property is the ability to agglutinate erythrocytes and other cell types and to induce mitosis in a certain class of lymphocytes. The quarternary structure of Con A is dependent on pH, temperature and salt concentration. At low pH, temperature and salt concentration a dimer is favored, while at high pH (7.2), high temperature (37°C) and high salt concentration (1.0 M) the tetramer predominates (3). In addition, the protein undergoes changes in tertiary structure as a consequence of metal ion and sugar binding (4,5), as detected by measurement of the circular dichroism. We report herein

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a conformational change not previously observed which is dependent on temperature and perhaps also on salt concentration, and which perturbs the binding of the sugar, methyl α-D-mannopyranoside (αMM). It was detected in the course of measurements of the heat of binding of that sugar.

MATERIALS AND METHODS

Con A was prepared from jack beans (Sigma) by the method of Agrawal and Goldstein (6) and gave a single band on electrophoresis on 5% polyacrylamide disc gels at pH 4.3 (7). Before use, the Con A was demetallized by acid treatment followed by remetallization with 2 mM Ca^{+2} and 2 mM Mn^{+2} (8). The Con A concentrations were determined by absorbance at 280 nm using A_{280} = 12.4 at pH 5.2 and 13.7 at pH 7.2 for a 1% solution (9). The buffers used at pH 5.2 and 7.2 were 0.05 M acetate and 0.05 M MOPS (Sigma), respectively. The sugar αMM was purchased from Sigma (Grade III) and used without further purification.

Calorimetric measurements were carried out at constant temperature ($\pm$ 0.001 C) using a modified Beckman 190C flow microcalorimeter (10,11). The calorimeter was calibrated by both the reaction of Tris with HCl (12) and by the heat of dilution of aqueous sucrose (13). The Con A solutions were equilibrated by dialysis against the buffer solution at the temperature of the measurement of binding for at least 24 hours. The αMM solutions were prepared by dissolution of αMM in the dialysis buffer. The two reacting components were mixed at a constant Con A to αMM volume ratio of 1:4. The desired mole ratios of protein to αMM were obtained by use of initial Con A concentrations in the range of 1.5×10^{-4} to 3.0×10^{-4} M. The heats of dilution of Con A and αMM were obtained by mixing buffer with the protein or sugar solutions. The heats of the reaction were much greater than the heats of dilution for ν values less than 0.9. These heats, which represented at most 8% of the observed total for both Con A and for αMM, were subtracted from the total observed heats. All measurements were made in triplicate.

The value of ΔH was determined by saturating the available Con A with αMM at initial concentrations of 0.2, 0.1 and 0.05 M. The observed heat of reaction may be used to calculate the equilibrium constant for binding of αMM when saturation is not attained, according to equation 1, the Scatchard equation (14), which, when expressed in terms of the experimental parameters, is converted to equation 2.

$$\nu/L = K - \nu K \qquad (1)$$

$$\frac{Q}{\Delta H[\alpha MM]_{total}\, n_{Con\ A} - Q[Con\ A]_{total}} = K - \frac{Q \cdot K}{n_{Con\ A}\, \Delta H} \qquad (2)$$

where the symbols are defined below:

ν = the fraction of ligand bound to total Con A monomers.

L = free concentration of αMM

Q = observed heat of reaction at fixed concentration of Con A

ΔH = enthalpy change per mole αMM bound
K = association constant
$[\alpha MM]_{total}$ = total concentration of αMM
$n_{Con\ A}$ = total number of moles of Con A monomers
$[Con\ A]_{total}$ = total concentration of Con A, in terms of monomers

Ultraviolet difference spectra of Con A were obtained using a Cary 14 spectrophotometer with an 0-0.1 slide wire and temperature-controlled cuvette holders.

RESULTS

Heats of binding to αMM to Con A were measured under the conditions specified in Table I. Each entry represents the average of two or more determinations. The reaction is clearly exothermic with a ΔH nearly independent of temperature, pH or salt concentration. Typical Scatchard plots based on equation 1 are shown in Figure 1. A ΔH of approximately -33 kJ/mole predicts a doubling of the association constant for a decrease in temperature of 15° under the conditions of the measurements. Such changes in K are not observed at pH 5.2 at either salt concentration, and only partially at pH 7.2 in 1.0 M NaCl in the 10°-25° interval. The calorimetric measurements are not complicated by contributions from heats of ionization of the buffers. Heats of binding of αMM measured at pH 7.2 in the absence of MOPS (ΔH = -39.3 kJ/mol) are indistinguishable from those obtained in its presence. In addition, saturation of Con A with αMM under similar conditions results in no change in pH.

The effect of salt concentration on the affinity of Con A for αMM is evident at pH 5.2, 25°, where nearly a doubling in K results from lowering the NaCl concentration from 0.5 M to 0.1 M.

It is interesting to note that the decrease in entropy consequent to αMM binding can nearly be accounted for by the loss of the entropy of mixing of the sugar in the molar standard state.

Because the data indicate a temperature-dependent conformation change at pH 5.2 (see Discussion below), difference absorption spectra were taken

TABLE I

SUMMARY OF RESULTS OF METHYL α-D-MANNOPYRANOSIDE BINDING TO CONCANAVALIN A

Experimental Conditions	Temperature °C	K_a	ΔG kJ/mole	ΔH_{cal} kJ/mole	ΔS_{cal} J/mole deg	$\Delta H_{V.H.}$ kJ/mole
pH 5.2 0.1 M NaCl	10.1	9,640 (580)[a]	-21.6 (.13)[b]	-31.7 (.96)[c]	-35.7 (3.47)[d]	0.9
	25.0	9,740 (880)	-22.8 (.25)	-35.2 (.29)	-41.7 (1.26)	
pH 5.2 0.5 M NaCl	25.0	5,300 (290)	-21.3 (.13)	-35.6 (.29)	-48.1 (3.81)	-6.3
	37.0	4,790 (80)	-21.8 (.04)	-38.5 (.29)	-54.0 (.96)	
pH 7.2 1 M NaCl	10.1	9,820 (730)	-21.7 (.17)	-34.1 (.88)	-43.5 (2.93)	-29.2
	25.0	6,080 (210)	-21.6 (.08)	-41.6 (.92)	-66.9 (2.93)	
	37.0	3,300 (500)	-20.9 (.42)	-38.6 (.04)	56.5 (1.26)	

[a] Listed errors are the calculated standard errors in the slope of Scatchard plots fitted to a straight line (18).

[b] Listed errors are half the range of ΔG calculated from the upper and lower error values in K.

[c] Listed errors are the standard error of ΔH obtained by saturation.

[d] Errors are the root-mean-squares of the errors in ΔG and ΔH divided by the absolute temperature.

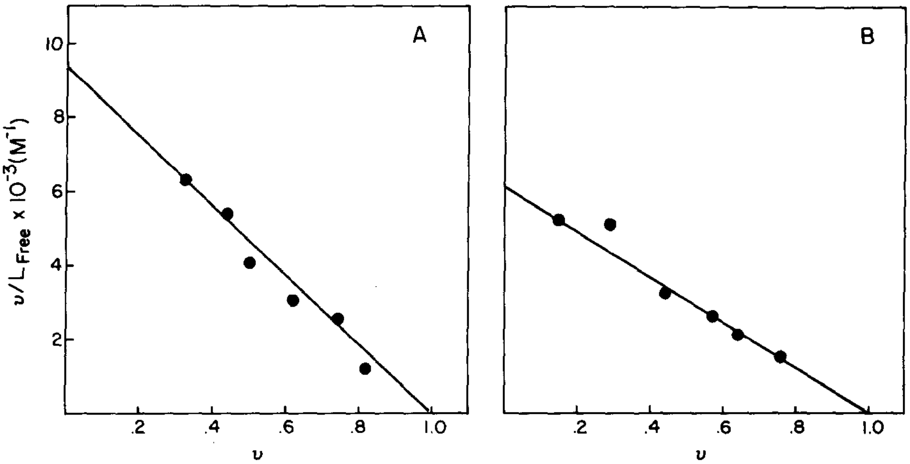

Fig. 1. (A) Scatchard plot showing binding of methyl α-D-mannopyranoside to Con A. (Con A) = 1.54 x 10^{-4} M, in 0.1 M NaCl, 2 mM Ca^{+2}, and 2 mM Mn^{+2}, 0.05 M acetate pH 5.2 and 25°C. The free αMM concentration is denoted by L_{free} and the fraction of sites occupied per Con A monomer is ν. (B) Scatchard plot showing binding of methyl α-D-mannopyranoside to Con A (Con A) = 1.40 x 10^{-4} M, 1.0 M NaCl, 2 mM Ca^{+2} and 2 mM Mn^{+2}, 0.05 M MOPS at pH 7.2 and 25°C.

at various temperatures at this pH. The results are presented in Figure 2. Differences are observed when the temperature of one Con A sample is varied and referenced to the spectrum of a second Con A sample maintained at 23.5°C.

DISCUSSION

Calorimetry has been used extensively in the analysis of ligand binding and conformation equilibria in biochemistry (15). A recent example is the analysis of the ricin-saccharide interaction (16). The remarkable feature of the data presented above is the difference between the observed ΔH and ΔH calculated from the variation of K with temperature. In the absence of artifacts of measurement, the implication is that changing temperature at pH 5.2 sufficiently alters the conformation of Con A, and thereby its affinity for αMM, to compensate for the effect of temperature on K predicted from the measured ΔH. A conformational change with an effect on K is also consequent to the change in NaCl concentration at pH 5.2. Such temperature and salt dependent changes in the tertiary structure of proteins are well known (17).

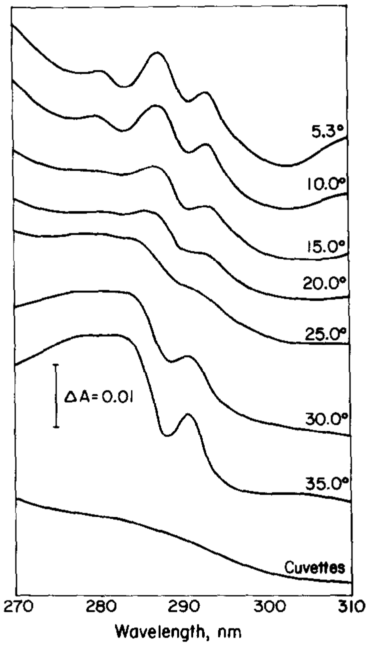

Fig. 2. Difference absorption spectra of Con A at various temperatures with reference Con A at 23.5°C. (Con A) = 5.4×10^{-5}, 0.5 M NaCl, 2 mM Ca^{+2}, 2 mM Mn^{+2}, 0.05 M acetate, pH 5.2.

In addition to the direct determination of enthalpy of binding, we have shown that calorimetry allows the direct determination of binding constants and eliminates the need for special ligands in competitive binding assays. This present study is now being extended to investigations on the binding of other saccharides to Con A.

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