SPECTRAL CHANGES ACCOMPANYING THE INTERACTION BETWEEN METAL LIGANDS AND CONCANAVALIN A

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1. Introduction

The lectin, concanavalin A (con A), is known to have at least four distinct binding sites. On site binds a transition metal, which, when occupied, activates a calcium binding site [1]. When both metal binding sites are filled, a saccharide binding site is activated. In addition, recent evidence suggests that con A binds a variety of non-polar compounds [2]. Nuclear magnetic resonance and high resolution X-ray crystallographic data [3–6] have shown that metal ion, saccharide and hydrophobic group binding sites are spatially distinct. Since aromatic residues are either coordinated to metals or are in close proximity to the metal binding sites [4,5] one might predict that the interaction of metal ligands with con A would create perturbations which could be detected spectrally. Spectral changes could then be used to study the binding and kinetics of the metal–con A interaction. In this report, we show that the metals associated with native con A, Mn^{2+} and Ca^{2+}, induce ultraviolet difference spectra in the lectin. Advantage is taken of these spectral changes to titrate con A with calcium.

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2. Experimental

Con A was purified according to Agrawal and Goldstein [7]. Metal-free con A was prepared by dialysis of the protein against 0.5 M NaCl, adjusted to pH 1.5–2.0 with HCl [1]. Metal-free con A would not precipitate glycogen unless both Mn^{2+} and Ca^{2+} were added to the solution. Atomic absorption analysis (Instrumentation Laboratory, Inc., Model 253) showed that native con A possessed 0.75 moles Mn^{2+} and 0.60 moles Ca^{2+} per mole of con A subunit (27 000 mol. wt.) [ref. 5]. Metal-free con A contained 0.012 moles Mn^{2+} and 0.16 moles Ca^{2+}, respectively, per mole con A subunit. Con A concentrations were determined spectrophotometrically, assuming that 1.0 mg/ml gives an absorbance of 1.14 at 280 nm with a 1 cm pathlength [8].

Difference spectral measurements were obtained by use of a Cary Model 15 spectrophotometer equipped with a 0–0.1 absorbance slidewire. All difference spectral studies were at room temperature using a dynode setting of 3 and a sensitivity adjustment of 2 on the Cary 15. Changing the dynode setting to 2 did not alter the spectra. Baselines were reproducible to within ± 0.005 absorbance units. For spectral measurements, con A was dissolved in 0.1 M NaCl, 0.04 M acetate buffer, pH 5.0. A subunit molecular weight of 27 000 daltons for con A was assumed in all calculations [5].

3. Results and discussion

Curve 1 of fig.1 shows that when Mn^{2+} is added to metal-free con A an ultraviolet difference spectrum is generated. The difference spectrum is characterized by major inflection points at 294, 287, 281, 271, 267, and 241 nm. When Ca^{2+} is added to the con A-Mn^{2+} solution a similar difference spectrum is observed, but of larger magnitude, and the 241 nm peak shifted to 244 nm (curve2, fig.1). The Mn^{2+} -induced difference spectrum is generated immediately upon mixing the metal with con A. The Ca^{2+}-induced absorbance changes, however, did not reach equilibrium for approximately 45 min. The Ca^{2+}-induced spectral changes could not be detected unless the metal-free con A had been previously mixed with Mn^{2+}. This provides further evidence that the transition metal site must be occupied before the calcium site is activated [1]. Substitution of Co^{2+}, Zn^{2+}, or Ni^{2+} for Mn^{2+} resulted in the same kinds of spectral changes shown on both curves.

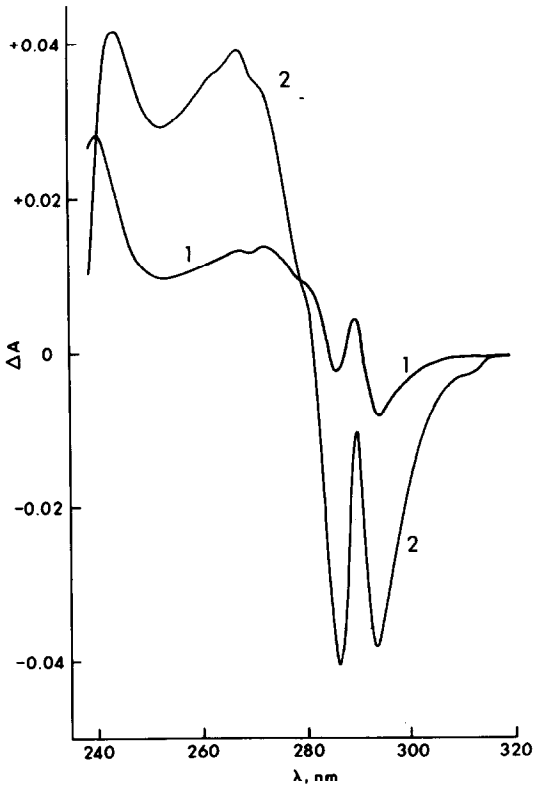

Fig.1. Metal ligand induced ultraviolet difference spectra in concanavalin A. The standard four cuvette arrangement was used. After baseline adjustment, $MnCl_2$ (10^{-3} M) was added to metal-free con A (8.84×10^{-5} M) in the 'Sample' compartment. The spectrum, shown by curve 1, was then scanned. Similarly, $CaCl_2$ (10^{-3} M) was added to the con A–Mn^{2+} solution and the resulting difference spectrum was obtained, shown by curve 2. The baseline was readjusted prior to addition of calcium. Changes in the concentration of metal-free con A resulted in proportionate changes in the magnitude of the difference spectra.

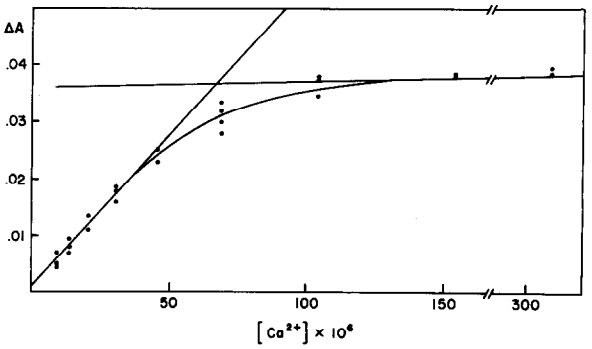

Fig.2. Titration of Mn^{2+}–con A with Ca^{2+}. The results include points from four separate titrations. Mn^{2+} concentration was 10^{-3} M. Con A concentration was 8.41×10^{-5} M. The points were derived from absorbance changes associated with the 287 nm peak.

The transition metal-induced absorbance changes were not sufficiently large to permit spectral titrations. However, it was possible to titrate con A-Mn^{2+} solutions with Ca^{2+} and determine the dissociation constant and number of binding sites. Results of four separate Ca^{2+} titration experiments are shown in fig.2. At the equivalence point, shown by the intersecting lines, it is seen that 0.79 moles Ca^{2+} combines with 1 mole con A subunit. The dissociation constant, K_d, for the Ca^{2+}-con A complex was calculated by the following expression

$$K_d = \frac{(1-\alpha)^2}{\alpha} \cdot [Ca^{2+}]_{eq}$$

where α is the fraction of Ca^{2+} sites occupied and is proportional to the absorbance change, *i.e.*, $= \triangle A/\triangle A$ equivalence point. Based on the foregoing equation and the data in fig.2 a value of $K_d = 1.9 \times 10^{-6}$ M was calculated. Kalb and Levitzki [1] reported a K_d

of approximately 3.3×10^{-4} for Ca^{2+}-con A binding. Our value is much lower. At the present, this discrepancy is not clear. Kalb and Levitzki used equilibrium dialysis which requires prolonged incubation times. Also, Ca^{2+} saturation of con A was not obtained in their studies. Our method requires about 3 hr. to obtain 10–12 spectra. Moreover, saturation is easily obtained using the spectral technique. In addition, the Scatchard plot of Kalb and Levitzki for the Ca^{2+}-con A complex was not linear, indicating heterogeneity of association constants. We have been unable to completely remove Ca^{2+} from the con A which probably reflects the value of 0.79 observed for the number of calcium binding sites. Spectral changes have great potential usefulness in studying metal ligand–con A interactions. For example, it should be possible to determine spectrally the kinetics of Ca^{2+}–con A binding because of the relatively lengthy time to reach equilibrium.

The spectral changes observed in the near UV region (fig.1) may reflect direct interactions between the metal and chromophore(s) which absorb at these wavelengths. The Mn^{2+} binding site appears to be associated with three side chain carboxyls and an histidine side chain [4,5]. The Ca^{2+} binding site, which is located 4.3 Å from the Mn^{2+} site, is associated with two of the side chain carboxyls which bind Mn^{2+}, a tyrosine side chain and an asparagine side chain [4,5]. Tryptophan and phenylalanine do not appear to participate directly in metal binding. The spectral changes we observe upon the inateraction between metals and con A (fig.1) suggests that perturbations of tryptophan, tyrosine, and possible phenylalanine and histidine have occurred. Metal-induced conformational changes would explain the origin of these difference spectra. Crystallographic studies also suggest a metal-induced conformational change in con A. Jack et al. [9] found that crystals of metal-free con A were characterized by different unit cell dimensions than crystals derived from native con A. The asymmetric unit weight of metal-free crystals was 52 000 daltons while that for native con A crystals was 26 000 daltons. Furthermore, the metal-free crystals could be converted into the native form by incubation in Mn^{2+} and Ca^{2+}. McCubbin et al. [10] found changes in the aromatic circular dichroism spectra of con A upon the sequential addition of Mn^{2+} and Ca^{2+} with the largest change associated with Ca^{2+} binding. Hardman and Ainsworth have pointed out that Mn^{2+} and Ca^{2+} stabilize the structure of con A by coordinating to the cluster of negative side chains in the metal binding sites [4]. In the absence of metals the side chains would separate by coulombic forces and expose the polypeptide chain to solvent [4]. Similarly, Edelman et al. [5] suggested that the sequential coordination of Mn^{2+} and Ca^{2+} to metal-free con A would cause structural alterations in the protein.

Acknowledgements

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