

pH-Dependent Conformational Changes of Concanavalin A

(Moffitt equation/ORD spectra/CD spectra/precipitin/protein structure)

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Communicated by James V. Neel, June 18, 1971

ABSTRACT The pH dependence of the conformation of concanavalin A has been studied by means of optical rotatory dispersion and circular dichroism spectroscopy. At pH 2.9, 5.0, and 7.0, the major contribution to organized structure appears to be the β conformation. At pH 9.1, the conformation of concanavalin A approaches the random coil or unordered form. No evidence could be found for the presence of any significant amount of α helix. The pH of maximum precipitin-like activity of concanavalin A is paralleled by the pH dependence of the parameter b_0 in the Moffitt equation.

The importance of protein conformation in protein-substrate interactions is often difficult to assess. Concanavalin A, the carbohydrate-binding protein (phytohemagglutinin) of the jack bean (1) has been shown to display many of the properties of immune antibodies. This protein gives typical precipitin-type curves with a select group of polysaccharides and glycoproteins (2-8), specifically binds low molecular weight carbohydrate ligands as revealed by equilibrium dialysis (9, 10) and inhibition of the precipitin reaction (4, 5, 7, 11-14), and forms typical precipitin bands on agar-gel diffusion and immunoelectrophoresis (8, 15-17). As such, concanavalin A can serve as an idealized model for the antibody-antigen system and, therefore, any conformational changes that occur in concanavalin A, could help in our understanding of the behavior of immune antibodies.

In recent years the use of circular dichroism (CD) and optical rotatory dispersion (ORD) measurements to evaluate the conformation of proteins has been developed to the point where such experiments are almost routine. These techniques are not without pitfalls, but the remarkable success in most correlations of ORD-CD analyses with x-ray diffraction studies of protein structure justifies the use of the method. The conformational analysis of concanavalin A was undertaken to establish (a) the role of conformation in the biological reactivity of this protein and (b) the existence of a possible correlation between the biologically reactive conformation of concanavalin A and the behavior of immune antibodies.

MATERIALS AND METHODS

Concanavalin A was prepared by the method of Agrawal and Goldstein (18). Solutions for ORD and CD measurements were brought to the desired pH by exhaustive dialysis against the appropriate buffer. Buffers were 0.1 M acetate, phosphate, or carbonate adjusted to the desired pH of 2.9, 5.0, 7.0, and 9.1.

Protein concentrations were determined by digestion with 7 N H₂SO₄ followed by ninhydrin analysis for ammonia (3).

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ORD and CD spectra were initially recorded on a JASCO ORD/CD-UV-5 instrument. Later, this instrument was modified to give the SS-20 configuration and equipped with a Nuclear Data prototype data acquisition system. ORD results are reported in terms of the mean residue rotation, $[m']$ at each wavelength, obtained from the specific rotation, $[\alpha]_\lambda$, by the usual relationship:

$$[m']_\lambda = \frac{3}{n^2 + 2} [\alpha]_\lambda \frac{115}{100} \quad [1]$$

The data above 350 nm were analyzed according to the equation of Moffitt and Yang (19):

$$[m']_\lambda = \frac{a_0 \lambda_0^2}{\lambda^2 - \lambda_0^2} + \frac{b_0 \lambda_0^4}{(\lambda^2 - \lambda_0^2)^2} \quad [2]$$

with λ_0 set equal to 212 nm.

Data collected after the instrument had been modified were processed on an IBM 360/67 computer; ORD data analyzed according to the Moffitt equations were also treated by computer and a least-squares program was used to fit the straight line to the data.

The ellipticity $[\theta]$ data were calculated from the equation:

$$[\theta] = 3300 \frac{\Delta A}{l \times c/115} \quad [3]$$

where l is in cm and c is in g/liter; CD data were not corrected for the refractive index dispersion of the solvent.

These experiments were run on three different preparations of concanavalin A in order to check reproducibility of the data and to eliminate from consideration a contribution to the CD spectra arising from light scattering and flattening-broadening due to particulate matter.

Ultraviolet spectra were obtained on a Cary 15 spectrophotometer.

RESULTS

ORD studies

The ORD spectra as a function of pH over the wavelength range 280-200 nm are presented in Fig. 1. A single trough at $\lambda = 228-229$ nm, with crossover at $\lambda = 220$ nm, characterizes the curves at pH 2.9, 5.0, and 7.0. At pH 9.1, the shape of the curve has been altered to give a broader trough at $\lambda = 226-227$ nm, and a crossover at $\lambda = 200$ nm. The mean residue trough rotation $[m']$ varies from -1300 at pH 2.9 and 7.0, to -1600 at pH 9.1, and -1800 at pH 5.0.

The Moffitt analysis of the ORD data above 350 nm is shown in Figs. 2 and 3. In dimethyl sulfoxide, a less-polar solvent than water, the slope of the line has clearly changed from the small negative values observed in aqueous media to a

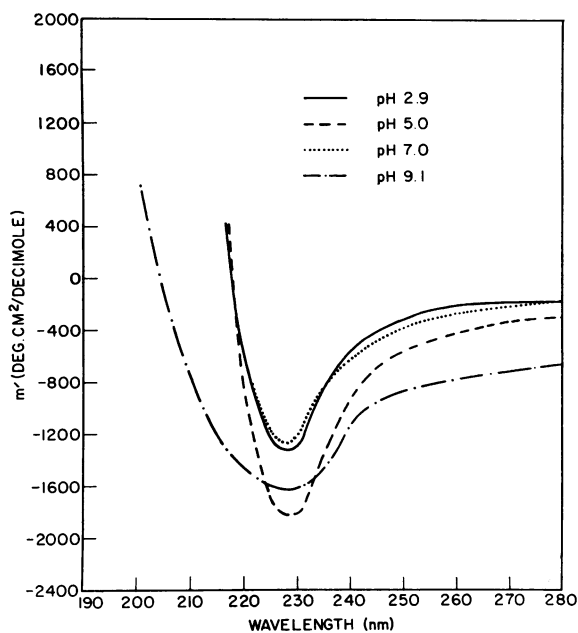


FIG. 1. The pH dependence of the ORD spectrum of concanavalin A.

positive value, indicating a large change in the conformation of concanavalin A. The values of a_0 and b_0 are presented in Table 1. If the parameter b_0 is plotted as a function of pH, a curve with a maximum in the profile is obtained (Fig. 4). The pH at which this maximum occurs lies in about the same pH range as that in which the maximum precipitating activity of concanavalin A toward glycogen, dextrans, α -mannans, etc. has been reported (3–5).

CD studies

The CD spectra, as a function of pH, covering wavelengths from 240–200 nm and 350–250 nm are presented in Figs. 5 and 6. At pH 2.9 and 5.0, the curves are characterized by a broad trough at 220–222 nm, with a slight shoulder at 210 nm and a crossover at 207 nm. At pH 7.0, the trough at about 220 nm is similar to the troughs at pH 2.9 and 5.0; however, the second shoulder is more pronounced and is blue-shifted to 206 nm, with the crossover now occurring at 202 nm. At pH 9.1, only a single intense trough at 212 nm is observed and the crossover now occurs at 199 nm.

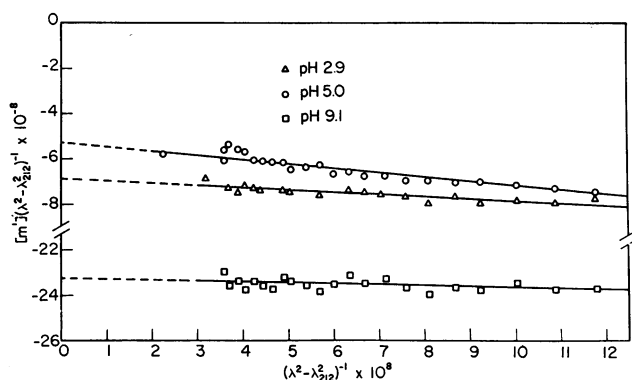


FIG. 2. Moffitt plots of the ORD data of concanavalin A at pH 2.9, 5.0, and 9.1.

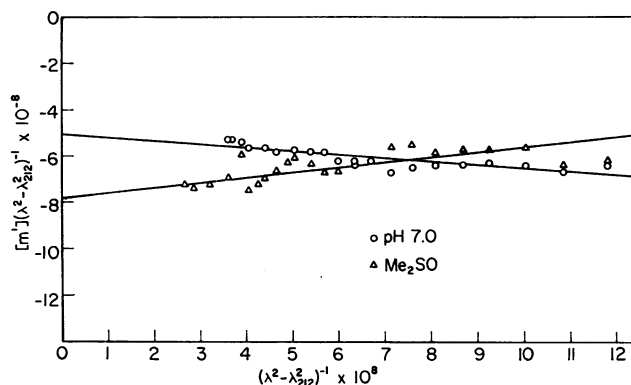


FIG. 3. Moffitt plots of the ORD data of concanavalin A at pH 7.0 and in dimethylsulfoxide.

The effect of pH on the Cotton effect of the aromatic side-chain is shown in Fig. 6. The most striking effect occurs at pH 9.1, with the complete inversion of the Cotton effects from the positive type displayed at all the lower pH values to the negative type at this pH.

UV spectra

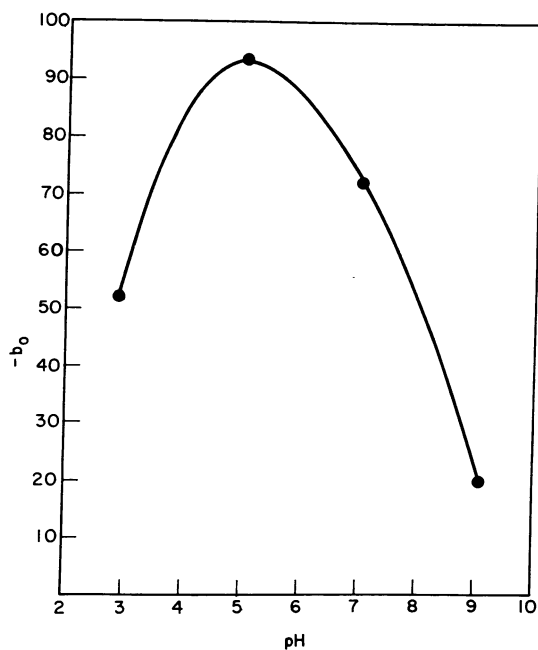
The effect of pH on the absorption spectrum of concanavalin A is shown in Fig. 7. Although these spectra exhibit a small pH-dependence, particularly in the 200-nm region and below, the effect is not as pronounced as in the ORD and CD spectra. Nevertheless, these spectra are of interest in their own right since they display a number of peaks and shoulders in the region from 300 to 250 nm that are not often observed in protein spectra.

DISCUSSION

The results of the present study demonstrate that, under our experimental conditions, the conformation of concanavalin A is strongly pH dependent. The observed dependence on pH of the organized structure corresponds fairly well with the pH dependence for maximum precipitation (3–5) with various carbohydrate polymers. Our analysis of the ORD and CD curves, in conjunction with the fitting of the data to the Moffitt equation, indicates that the helical content of this protein is very low or may be totally lacking. However, the shapes of the curves and the wavelengths at which the Cotton effects occur rule out a completely random-coil structure. The assignment of the characteristic wavelengths for the CD of unordered polypeptides and proteins has recently been re-investigated (20). ORD Cotton effects arising from the unordered conformation are troughs at 238 and 204–205 nm, crossover at 198 nm, and a peak at 228 nm (21). The original CD Cotton effect characteristic of the unordered conformation is a trough in the region of 196–202 nm (22). The more recent assignment of CD parameters for the unordered structure

TABLE 1. Moffitt data on concanavalin A

pH	Buffer	a_0	b_0
2.9	Acetate	-15.14	-52.13
5.0	Acetate	-11.80	-94.31
7.0	Phosphate	-11.14	-72.03
9.1	Carbonate	-51.78	-19.8
	Me ₂ SO	-17.37	+112.2

FIG. 4. The pH dependence of b_0 .

indicates that the single negative trough can occur anywhere over the region from 200 to 220 nm. The observed ORD and CD Cotton effects for concanavalin A are sufficiently different from the typical Cotton effects of the unordered structure to eliminate this conformation as the dominant one in concanavalin A over the pH range 2.9–7.0.

There appear to be pH-associated conformational changes that can take place in concanavalin A, occurring between pH 6.0 and 9.0. Since the isoelectric point of concanavalin A is reported to be at $\text{pH } 7.1 \pm 0.1$ (23), it is not surprising that, in going from the positively charged protein to a protein approaching zero net charge, the conformation of the protein changes. As the charge is again built up at pH values greater than 7, electrostatic effects again will influence the structure and a new conformation may be formed.

The binding of specific low molecular weight substrates to concanavalin A occurs over a broad range of pH (pH 3–8), including pH regions where precipitation does not occur (pH <5 and >7) (24). Since this binding appears to involve charge-dipole and hydrogen-bond interactions (3, 12, 25, 26), the binding of substrates in the region of the isoelectric point of the protein may further reduce the number of charges on the protein. This factor, in conjunction with the conformational change of the protein, which conceivably has removed polar sites from exposure to the solvent medium, could result in a reduced solubility for the concanavalin A-substrate complex in the pH region of 6.0–7.0.

Our data and observations would appear to be in greater accord with the assignment of the β -conformation as the major organized structure in concanavalin A than with any other alternative possibilities. In addition, the content of β structure is pH- and solvent-dependent. The deviations of the observed minima from the normal wavelength at which β -structure minima occur is ascribed to contributions from the aromatic chromophores. An ORD study of concanavalin A by Doyle *et al.* (26) was interpreted as indicating the presence of about 7.5% helical structure. We consider their interpretation to be

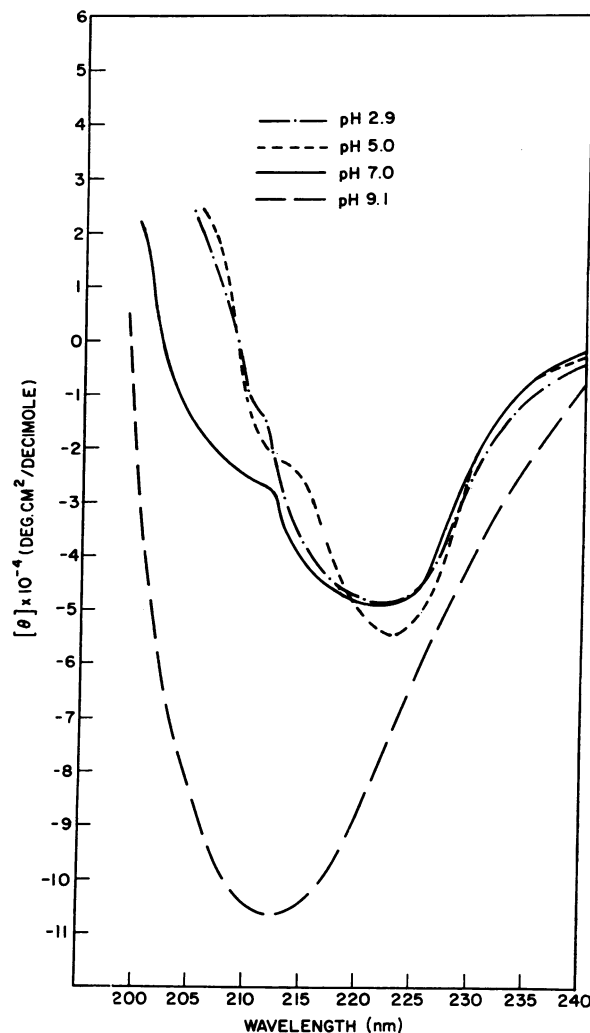


FIG. 5 The pH dependence of the CD spectrum of concanavalin A over the wavelength region 240–200 nm.

in error, inasmuch as a negative value for b_0 having such small magnitude is not a sufficient criterion for the presence of α -helical content. In a CD study of concanavalin A at a single pH value, Kay (27) found that concanavalin A gave spectra that could be interpreted as indicating the presence of β -structure.

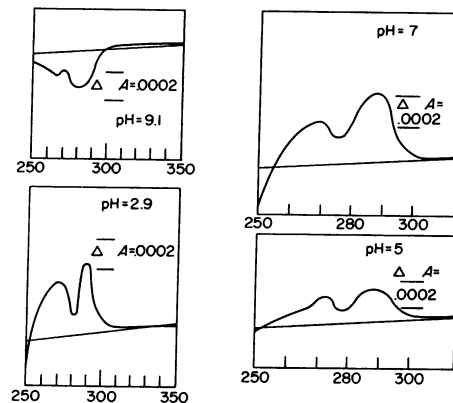


FIG. 6. Aromatic side-chain Cotton effects of concanavalin A as a function of pH. The abscissas are wavelength, in nm; the ordinates are absorbances.

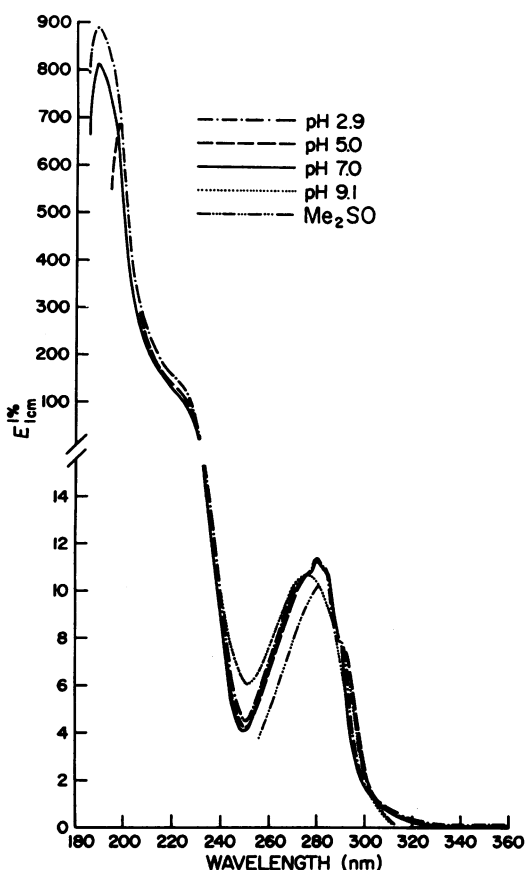


FIG. 7. Effect of pH on the UV spectrum of concanavalin A.

Values for b_0 that are quite low have been reported for polymers in films having a known content of β conformation. In a study of poly(D,L alanine) containing 67% D-aminoacyl residues, infrared evidence for the presence of the β conformation was obtained by Elliott *et al.* (28). Similar behavior was observed in films of *Bombyx mori* silk. Both of these polymers had very low b_0 values. Wada *et al.* (29) have shown that the β conformation also displays a complex dispersion that fits the Moffitt equation. A change in b_0 from negative to positive can sometimes be taken as evidence for a change in the helical sense of the polymer (30, 31). Since we find no evidence for the presence of any helix content, this interpretation of our data is not tenable.

It is sometimes possible to enhance the content of β structure of proteins and polypeptides by using organic solvents that are less polar than water (32). This enhancement is reflected in a change of b_0 from a small negative value to a larger positive value. Dimethyl sulfoxide as a solvent for concanavalin A gave us a result that conforms exactly to the behavior that is described in the literature for such systems (32).

It is also important to point out an alternative possibility; namely, that if a helix-coil transition were occurring as a function of pH, then a plot of b_0 against pH would have yielded a sigmoid curve instead of a bell-shaped curve (32, 33). Nearly all transitions of the helix-coil type, whether accomplished by changes in solvent polarity, pH, or temperature, are centered about a distinct critical point and exhibit a relatively sharp sigmoidal shape (33). Therefore a helix-coil interpretation of our results would seem to be ruled out.

Considering the analogy that exists between concanavalin A-polysaccharide interaction and the antibody-antigen interaction, we suggest that the role of β structure in the conformation of immunoglobulins deserves further attention.

Portions of this work were reported at the 156th Meeting of the American Chemical Society, Atlantic City, N.J., September 1968. This research was supported in part by U.S. Public Health Service Grants NB-05306 and AM-10171 from the National Institutes of Health.

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