THE PRESENCE OF β-STRUCTURE IN CONCANAVALIN A

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1. Introduction

The present communication reports the results of an ultraviolet circular dichroism (CD) examination of concanavalin A (con-A), the crystalline saccharide binding protein of Jack bean. In the native state, the protein shows wavelength characteristics usually attributed to the β-form. Upon the addition of sodium dodecyl sulfate (SDS), it probably exists as a mixture of α-helix and random coil. Con-A also possesses a distinct fine structure in the aromatic region, which arises largely from asymmetric interactions involving tryptophan residues.

2. Experimental

CD measurements were made with the Cary model 6001 recording spectropolarimeter calibrated with d-10-camphor sulfonic acid. All reported studies were at 29° and at an absorbance of less than 2. Cells of 1 cm and 0.1 mm path length were used in the near ultraviolet (250–300 nm) and far ultraviolet (190–250 nm) regions, respectively. CD data are presented as mean residue ellipticity $[\theta]$ in degrees × cm^2 × $decimole^{-1}$. The mean residue weight employed for con-A was 108. Signal-to-noise ratios at band maxima were frequently as high as 25:1, and they were never lower than 10:1. The errors in ellipticity are estimated to be 10% in the aromatic region, 2% at 210–225 nm and 7% at 190–200 nm.

Concanavalin A was prepared from Jack bean meal (Sigma Chemical Co., St. Louis) by crystallization, as previously described [1]. Circular dichroic spectra of this native protein were indistinguishable from those of demetalized con-A, prepared by dialysis of acidified protein against twice distilled water, as described previously [2]. Since con-A represents a pH-sensitive aggregating protein system [3], CD measurements were confined to pH 5.2 (0.02 M sodium acetate buffer with 0.2 M NaCl), where the protein has been demonstrated to be monomeric (molecular weight, 55000) by sedimentation equilibrium analysis [3]. The $E^{1\%}_{1cm}$ value at 280 nm for the protein in this solvent system is 12.4 [4].

3. Results and discussion

Fig. 1 presents the far ultraviolet CD spectrum of con-A in the native state, as well as in the presence of either 5×10^{-3} M SDS or 5 M guanidine HCl. Under native conditions, the experimental curve has a maximum at 197.5 nm with an ellipticity of 13200°, a crossover at 207.5 nm, and a minimum at 222.5 nm with an ellipticity of −7800°. This spectrum is reminiscent of the β-form, which under normal circumstances is the summation of a positive $\pi^{\circ}-\pi^{-}$ band at 195 nm, with a crossover at 205 nm and a negative $n-\pi^{-}$ band near 217–219 nm [5]. With con-A, the positions of the maximum and crossover fit β-structure characteristics; however, there is a 3–4 nm displacement to higher wavelengths in the $n-\pi^{-}$ band. The nature of the interactions causing this shift are not known but it has been suggested, on the basis of its occurrence with other β-structured polypeptides, that side-chain interactions and steric effects due to side-

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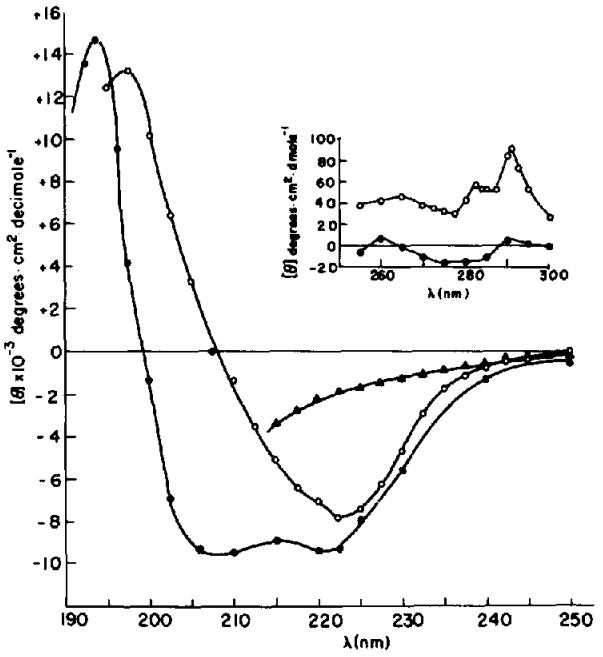

Fig. 1. Circular dichroic spectra of concanavalin A in: 0.05 M acetate buffer, pH 5.2 + 0.2 M NaCl (○—○); 0.05 M acetate buffer, pH 5.2 + 0.2 M NaCl and 5 × 10^{-3} M SDS (●—●); 5 M guanidine hydrochloride (▲—▲).

Inset, circular dichroism of the aromatic residues in concanavalin A in 0.05 M acetate buffer, pH 5.2 + 0.2 M NaCl (○—○) and in 0.5 M acetate buffer + 0.2 M NaCl and 5 × 10^{-3} M SDS (●—●).

chain bulkiness may cause small variations in the orientation of the transition moments of the β-structure backbone chain [6].

It would also appear that there is very little α-helix in native con-A, since there is no evidence in the CD spectrum for the presence of a second negative band or shoulder at about 206 nm ($\pi-\pi^-$ transition), normally displayed by even small amounts of the helical form [7]. The magnitude of the ellipticities indicate that con-A is not entirely in the β-form, although it is notable that θ_{222} is approximately 50% of the value recorded for the β-form of polylsine [8]. However, for lack of enough data about relevant reference compounds, it is premature to try to predict the fraction of the protein that has adopted the pleated-sheet conformation.

As anticipated, in 5 M guanidine HCl, con-A assumes the random coil conformation. However, addition of 5 × 10^{-3} M SDS to a solution of native con-A results in the development of a CD spectrum indicative of the formation of some α-helix. In particular, the appearance of a second negative ($\pi-\pi^-$) dichroic band at 206 nm, the increase in the intensity of all the bands, and the shift of the positive maximum from 197.5 to 193.5 nm reflect this phenomenon. Since the wavelength positions of the dichroic bands, including crossover, are now characteristic of the α-helical form [9], one may conclude that the CD spectrum of con-A in this medium does not suggest the presence of much pleated-sheet structure. It is also noteworthy that the positive band at 193.5 nm exhibits an intensity smaller than would be expected relative to that of the negative bands. This suggests the presence of randomly coiled sections in the polypeptide chains, which are known to display a negative band around 195 nm [10, 11]. Since the same qualitative argument pertains to the native protein as well, one wonders whether the aperiodic but organized regions in the native structure remain the same after SDS treatment, or whether truly random sections exist then.

The present study does not shed new light on the mechanism of SDS action; however, in qualitative terms it suggests that the action of SDS upon con-A leads to destruction of probably most of the native β-structure and formation of some α-helix. Unfortunately, it is not possible to decide whether one conformational form derives from the other, or from other regions of the native protein. It is noteworthy that SDS has been shown to increase the apparent α-helical content of several other protein systems [12–16].

The CD spectrum of native con-A in the aromatic region (inset, fig. 1) consists of three principal positive bands at 291, 282.5 and 265 nm, with ellipticities of 40–100 deg cm^2 $decimole^{-1}$, i.e., two orders of magnitude smaller than the values associated with the 190–230 nm bands. The last band is very broad, and probably represents more than one transition. The positions of the three bands are consistent with a mutually non-random arrangement of tryptophan (6/mole protein [17]), being at the same wavelengths and possessing the same sign as the CD bands observed in polytryptophan films [6]. The lower wavelength absorption could conceivably reflect order in tyrosine residues as well [18]; however, since there are no disulfide bridges in con-A, the large rotatory strength of the cysteinyl chromophore need not be considered.

Disruption of the native structure by the addition of 10^{-3} M SDS results in a marked reduction of the optical activity in the aromatic region, and the long wavelength Cotton effects all but disappear. Some structure however remains, which may or may not still involve tryptophan, and is presumably associated with the residual or newly formed structured part of the chain (α-helix), because it vanishes completely in 5 M guanidine HCl.

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