

Short Communication

Polyproline II Confirmation in the Protein Component of Arabinogalactan-Protein from *Lolium multiflorum*¹

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ABSTRACT

Circular dichroism spectra of the arabinogalactan-protein from suspension-cultured *Lolium multiflorum* (ryegrass) endosperm cells demonstrate the presence of polyproline II conformation in the protein moiety of the proteoglycan. Subject to a number of theoretical and practical constraints of the method, it can be estimated that at least 30% of the protein component is in this conformation.

Hydroxyproline-rich proteins are widely distributed in higher plants, where they are found as constituents of arabinogalactan-proteins, cell wall glycoproteins, and in certain lectins of the Solanaceae (11). The hydroxyproline-rich cell wall glycoprotein from carrot has been shown by circular dichrometry to exist in the polyproline II conformation (17), which consists of a left-handed helix of three residues per turn and a rise for residue of 0.31 nm (15). Based on optical rotary dispersion measurements, it has been suggested that lectins of the Solanaceae also contain polyproline II conformation (1, 7).

In contrast, there is no information on the conformation of the protein component of arabinogalactan-proteins, although it has been observed that only substrates capable of forming a polyproline II helix are hydroxylated by the peptidyl prolyl hydroxylase involved in arabinogalactan-protein biosynthesis in suspension-cultured ryegrass (*Lolium multiflorum*) endosperm cells (6). This has led to the suggestion that the protein moiety of the ryegrass arabinogalactan-protein might have regions of polyproline II conformation (6). Here we use circular dichroism spectra to provide evidence for this.

MATERIALS AND METHODS

Purification of Arabinogalactan-Protein. Liquid suspension-cultures of ryegrass (*Lolium multiflorum*) endosperm cells were maintained in the dark at 28°C on a modified White's medium containing 4% (w/v) sucrose (16). The arabinogalactan-protein

was isolated from the medium by affinity chromatography on myeloma protein J539-Sepharose (3). Its monosaccharide composition, mol wt, polysaccharide structure, and amino acid composition were the same as the preparation isolated by Anderson *et al.* (2). Detection of a single peak of density 1.66 g/ml after equilibrium ultracentrifugation on a CsCl density gradient (10) indicated that the preparation contained no free protein contaminants.

Circular Dichrometry. To examine the conformation of the protein moiety in the native and 'denatured' forms, circular dichroism spectra were measured under nitrogen at 20°C and 75°C using a Jasco-Durrum J-20 spectropolarimeter calibrated as described previously (18). The protein concentration in each sample was calculated from the hydroxyproline content (8) and amino acid composition (15% mol/mol hydroxyproline) (2). The error in the protein determination was ±3%. Data are expressed as mean amino acid residue ellipticity, $[\Theta]$, in degrees·cm²·dmol⁻¹.

To compare the spectrum obtained at 20°C with that at 75°C it is necessary to correct for the effects of broadening of the circular dichroism bands, evaporation, and expansion of the cuvette on heating. The magnitude of these effects was determined by comparing spectra of a solution of D-camphor-10-sulfonic acid in water at 20°C and 75°C (5). Differences in the two spectra were within experimental error (±5%).

RESULTS AND DISCUSSION

The circular dichroism spectrum of the ryegrass arabinogalactan-protein at 20°C shows a single trough at 200 nm ($[\Theta]$ -21,000) and an inflection at about 220 nm (Fig. 1); the latter suggests there is a minor band at about 220 to 225 nm ($[\Theta]_{222\text{ nm}}$ -3,300). The circular dichroism of the arabinogalactan chains does not interfere with the peptide circular dichroism in the 195 to 240 nm region (12, 17).

Since only extended, helical conformations of the polyproline II type and unordered protein conformations exhibit negative ellipticity near 200 nm (9, 13), it is likely that in the arabinogalactan-protein these conformations predominate over other ordered structures such as α -helices, β -sheets, and β -turns, all of which exhibit positive ellipticity at 200 nm (4).

Extended protein structures of the polyproline II conformation can be distinguished from unordered structures by the magnitude of the negative ellipticity in the 200 nm region. Thus, most denatured conformations have a minimum value for $[\Theta]$ of -10,000 to -12,000 degrees·cm²·dmol⁻¹ (9) while proteins containing a high proportion of polyproline II conformation show an ellipticity of approximately -45,000 to -50,000 degrees·cm²·

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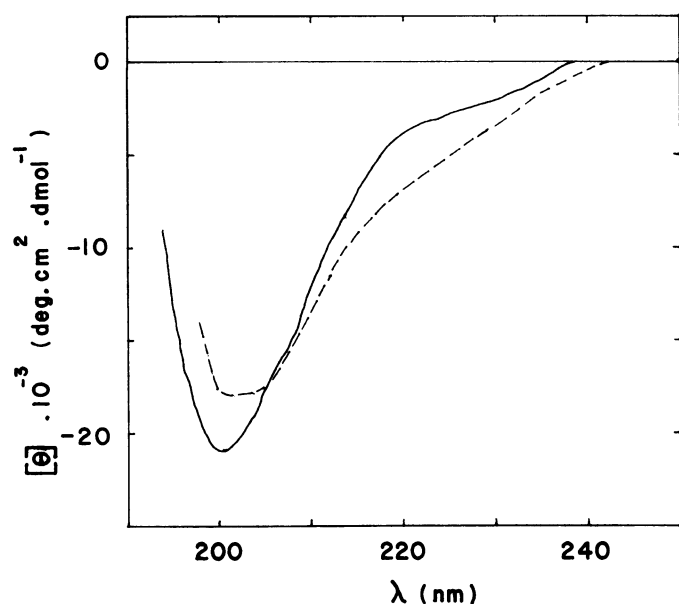


FIG. 1. Circular dichroism of arabinogalactan protein at 20°C and 75°C. Spectra of arabinogalactin protein in water recorded at 20°C (—) and at 75°C (---).

dmol⁻¹ (14, 17).

There are a number of difficulties and limitations in assessing the conformation of proteins containing polyproline II helices from circular dichroism spectra (12). However, using the value of $[\Theta] -11,300$ for denatured collagen as a reference for unordered structures (14) and a value of $[\Theta] -46,000$ as a reference ellipticity for polyproline II conformation (17), it can be estimated that the minimum ellipticity of $[\Theta] -21,000$ for the protein from arabinogalactan-protein (Fig. 1) corresponds to about 30% of the protein in the polyproline II conformation. If the arabinogalactan-protein contains α -helical, β -sheet, or β -turn conformations, which would contribute positive ellipticities at 200 nm, the content of polyproline II helix will be higher than 30%. Indeed, the absence of the characteristic positive ellipticity at 220 to 230 nm for polyproline type II helices (12, 14) and the inflection in the curve at 220 nm (Fig. 1), may be due to the negative ellipticities of other types of ordered structures in this region (4, 12). However, the data do not allow an identification of these possible conformations.

The spectrum of the arabinogalactan-protein recorded at 75°C shows an increase in the ellipticity in the 200 nm region to $[\Theta] -18,000$, a small shift of the minimum to longer wavelengths, and a decrease in ellipticity at about 200 nm when compared with the spectrum recorded at 20°C (Fig. 1). Each of these changes is observed during the transition from an extended polyproline II conformation to an unordered structure at elevated temperatures in the carrot cell wall glycoprotein (17), poly(glycyl-prolyl-alanine) (5) and collagen (14). However, the ellipticity at 200 nm does not increase to the values of $-10,000$ to $-12,000$ degrees-

cm² · dmol⁻¹ reported for fully denatured proteins. The apparent resistance of the polyproline II conformation of complete denaturation at 75°C has also been observed with the carrot cell wall glycoprotein and with synthetic poly(hydroxyproline) (17).

The circular dichroism spectrum for the ryegrass arabinogalactan-protein measured at 20°C (Fig. 1) is very similar to that obtained for the major cell wall glycoprotein from *Chlamydomonas reinhardtii* (12), indicating that protein conformation is similar in both. However, the spectrum differs significantly from that obtained with the carrot cell wall glycoprotein (17).

Interpretation of the circular dichroism data for the ryegrass arabinogalactan-protein in terms of polyproline II conformation in the protein component of the macromolecule is consistent with the observation that the peptidyl prolyl hydroxylase involved in its biosynthesis is specific for synthetic peptides containing the same conformation (6).

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