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The structure of C hordein

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The intrinsic viscosity of C hordein was determined in 70% (v/v) aqueous ethanol (± 10 mM NaCl) and 0.1 M acetic acid at temperatures between 15.3 and 50°C. Calculations based on the assumption (supported by previously published results) that C hordein has an ordered conformation indicated that the molecule is rod shaped, with molecular dimensions varying from about 360 × 17 Å to 265 × 20 Å. Circular dichroism spectroscopy indicated a secondary structure rich in β-turns, with some conformational differences in the two solvents and at different temperatures. The results are compatible with C hordein having a helical secondary structure based on repetitive β-turns.

Hordein *Molecular dimensions* *Secondary structure* *β-turn*

1. INTRODUCTION

C hordein is a group of storage proteins present in barley seed [1]. It is characterized by an unusual amino acid composition, with about 40 mol% glutamine, 30 mol% proline and 9 mol% phenylalanine. It has no cysteine, and hence no disulphide bonds, and less than 5 mol% of charged residues. Almost all of the residues, which total about 440, are present as repeated sequences, mostly octapeptides but also some pentapeptides [2,3]. Computer prediction and circular dichroism (CD) spectroscopy show the presence of an unusual secondary structure, with no α-helix or β-sheet, but regularly repeated β-turns reflecting the primary structure [2]. Further evidence for a regular β-turn-rich secondary structure comes from ^{13}C NMR [4].

In this paper we report further studies of the structure of C hordein, which show that its dimensions are consistent with the presence of a regular helix based on repetitive β-turns.

2. MATERIALS AND METHODS

Hordein was prepared from the mutant barley line Risø 56 as described [2,3]. Although a number of polypeptides are present they show a high degree of structural homology [5,6] and can be treated as a single protein for physico-chemical analysis.

Viscosity measurements were made in an Ostwald capillary viscometer with a flow time for water at 20°C of about 350 s. Solutions of C hordein in 0.1 M acetic acid, 70% (v/v) ethanol, 70% (v/v) ethanol/10 mM NaCl and 5.9 M guanidine hydrochloride were filtered through a 0.45 μm filter before use. Density measurements were made using a 10 ml pycnometer bottle. Protein concentrations were calculated from Kjeldahl nitrogen determinations (using a factor of 5.7 for the conversion of N to protein) for solutions in acetic acid and ethanol, or for solutions in guanidine hydrochloride, from optical absorption measurements and published extinction coefficients [7]. Partial specific volumes were calculated [8] from both the amino acid composition [1] and density measurements of C hordein solutions in 70% (v/v) ethanol and 0.1 M acetic acid. CD spectra were determined as described [2].

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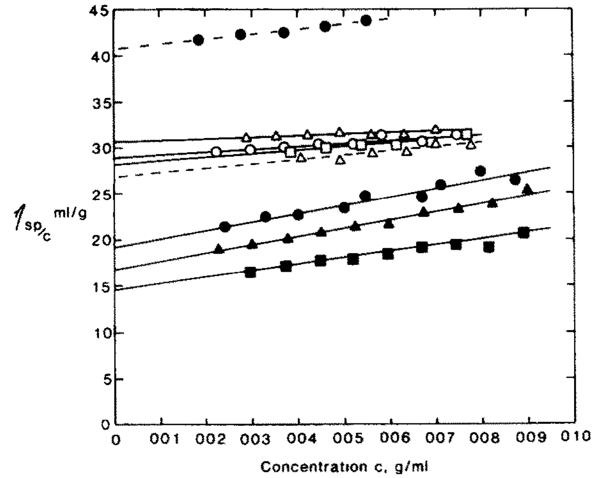

Fig.1. The reduced viscosity of C hordein in 0.1 N acetic acid and 70% (v/v) ethanol at a range of temperatures, and in 5.9 M guanidine hydrochloride and 70% (v/v) ethanol/10 mM NaCl. (●—●) 0.1 M acetic acid, 15.3°C; (▲—▲) 0.1 M acetic acid, 30°C; (■—■) 0.1 M acetic acid, 50°C; (○—○) 70% (v/v) ethanol, 20°C; (△—△) 70% (v/v) ethanol, 30°C; (□—□) 70% (v/v) ethanol, 50°C; (△---△) 70% (v/v) ethanol/10 mM NaCl, 30°C; (●---●) 5.9 M guanidine hydrochloride, 25°C.

3. RESULTS AND DISCUSSION

The reduced viscosities (η_{sp}/c) of C hordein under denaturing conditions (5.9 M guanidine hydrochloride), in 70% (v/v) aqueous ethanol/10 mM NaCl, and in 70% (v/v) aqueous ethanol and 0.1 M acetic acid at a range of temperatures are shown as a function of concentration in fig.1. The intrinsic viscosities determined by extrapolation to zero concentration are given in table 1. The experimentally determined partial specific volumes of C hordein in 70% (v/v) ethanol and 0.1 M acetic acid were both in close agreement with that calculated from compositional data (0.719 ml/g).

The values obtained for the intrinsic viscosities $[\eta]$ of C hordein in 70% ethanol and 0.1 M acetic acid are clearly incompatible with the protein possessing a compact globular configuration in these solvents ($[\eta]$ being in the range 3.3–4.0 ml/g for globular proteins [8]) and indicate that the protein exists as either highly solvated randomly coiled chains or relatively rigid asymmetric particles. In the case of the solutions in acetic acid, the degree of expansion of the polypeptide chain due to a polyelectrolyte effect is unknown. Unfortunately, it proved impossible to dissolve the protein in lower concentrations of acetic acid, and any attempt to increase shielding of charged groups by increasing the ionic strength of the solvent led to immediate precipitation. In aqueous ethanol the polyelectrolyte effect would be far less marked and, in fact, addition of NaCl to 10 mM had almost no effect on the value of $[\eta]$ (table 1).

Table 1

The intrinsic viscosity and calculated molecular dimensions and radius of gyration of C hordein

Solvent	Temperature (°C)	$[\eta]$ (ml/g)	Molecular dimensions			Radius of gyration (Å)		
			Length (Å) (L)	Diameter (Å) (d)	(L/d)	1	2	3
0.1 M acetic acid	15.3	19.31	301	18.5	16.3	86.9	62.2	70.3
	30.0	16.84	282	19.1	14.8	81.4	59.5	67.1
	50.0	14.68	265	19.7	13.5	76.5	56.8	64.1
70% (v/v) ethanol	20.0	28.98	352	17.3	20.3	101.6	71.3	80.5
	30.0	30.62	363	17.0	21.4	104.9	72.6	81.9
	50.0	28.05	350	17.2	20.3	101.0	70.4	79.5
70% (v/v) ethanol/ 10 mM NaCl	30.0	26.79	346	17.4	19.9	100.0	69.3	78.3
5.9 M guanidine hydrochloride	25.0	40.81	–	–	–	–	–	–

(1) Calculated assuming a rod-shaped molecule. (2,3) Calculated assuming a random coil conformation in ideal and non-ideal solvents, respectively

The following calculations on the shape and size of C hordein in solution are based on the assumption that the protein exists as an asymmetric hydrodynamic particle rather than in the form of a random coil. This assumption is supported by the relative insensitivity of $[\eta]$ to differences in temperature and by previously published results [2,4] showing the protein to have an ordered conformation in aqueous ethanol and 0.1 M acetic acid. Except where otherwise stated the relationships used are taken from Tanford [8].

The Simha shape factor, V, was calculated from the relationship

$$[\eta] = V(\bar{v}_2 + \delta_1 v_1^\circ) \quad [9]$$

where $\bar{v}_2$ is the partial specific volume of C hordein, v_1° the specific volume of the solvent and δ_1 the effective solvation of the macromolecule (taken as 0.2 g/g dry protein). From V the axial ratio (a/b) of the prolate ellipsoid which best represented the real molecule was obtained [8–10]. The values of the semi-axes a and b were then calculated using the relationships

$$V_\mathrm{h} = \frac{M}{N}(\bar{v}_2 + \delta_1 v_1^\circ)$$

and

$$V_\mathrm{h} = \frac{4}{3}\pi ab^2$$

where V_h is the hydrodynamic volume of the particle and M the unsolvated M_r, previously determined by sedimentation equilibrium ultracentrifugation in 6 M urea to be 52570 [1]. The M_r of 54300 calculated from the intrinsic viscosity [11] of C hordein in 5.9 M guanidine hydrochloride compares well with this value.

If C hordein is considered as a rod-shaped molecule of length L and diameter d, rather than as a prolate ellipsoid, then $L = 2a$ and $d = (2/3)^{1/2}(b/a)L$. Calculated values for L and d are given in table 1.

Although, as is usual, a value for δ_1 of 0.2 g/g has been assumed, the validity of this assumption for a solvent such as 70% (v/v) ethanol is less certain than for normal aqueous solvents. However, even doubling δ_1 to 0.4 g/g has a relatively minor effect on the calculated dimensions of the protein, decreasing L from 363 to 350 Å and increasing d from 17 to 19.3 Å for 70% ethanol at 30°C.

The values obtained for the axial ratios of C hordein in aqueous ethanol solvents can be used to test the validity of the initial assumption that the protein exists as an asymmetric hydrodynamic particle. A number of volume-independent shape functions have been computed which are more sensitive to asymmetry than the original β-factor of Scheraga and Mandelkern [12]. One such function, $K_s/[\eta]$ (the ratio of the coefficient of concentration dependence of the reciprocal sedimentation coefficient to the intrinsic viscosity) has been shown to have a value of 1.6 for random coils but of only about 0.4 for a particle with the degree of asymmetry calculated for C hordein [13,14]. The values of $[\eta]$ in table 1 lead to values for K_s of 43–49 and 10–12 ml/g from these $K_s/[\eta]$ values for the two extremes of structure. Although K_s was not determined experimentally in the present instance, the analogous K_η is easily obtained from the data in fig.1, and has values in the range 5–17 ml/g (average 11.7 ml/g) for the ethanol solutions. As K_s for a simple non-associating solvent is always less than K_η [13,14], the random coil model can be excluded.

Also shown in table 1 are values for the radius of gyration, R_G, of C hordein, calculated on the basis of a rod-shaped molecule ($R_\mathrm{G} = L/\sqrt{12}$) and of a flexible coil conformation ($R_\mathrm{G} = \sqrt[3]{(3M[\eta]/10\pi N\xi^3)}$), where ξ is taken as 0.875 in ideal solvents and 0.775 for poor solvents. R_G is a parameter which can also be determined experimentally by light scattering and comparison of computed and measured values would provide further proof of an asymmetric or flexible coil conformation.

The results in table 1 show that C hordein has a less extended conformation in 0.1 M acetic acid than in 70% ethanol, and that increasing temperature results in an increasingly compact conformation in the former solvent only. These differences may result from the effects of temperature and solvent on the strength of hydrogen bonds and hydrophobic interactions stabilizing the conformation. The latter is expected to be favoured and to show a marked increase in intensity on heating in dilute acetic acid, but not in aqueous ethanol.

The presence of some differences in the conformation of C hordein in 70% ethanol and 0.1 M acetic acid, and at different temperatures, is confirmed by analysis of the secondary structure by

CD spectroscopy (fig.2). The CD spectra in 70% ethanol have been detailed in [2]. The far-UV spectrum at 10°C shows a strong negative peak at 203 nm and a shoulder at 230 nm. The latter is absent in 4 M urea, which also results in loss of two strong vibronic components, due to phenylalanine side chains in a fixed conformation, in the near-UV spectrum (not shown). The spectra have been interpreted as indicative of a conformation rich in β-turns, with no α-helix or β-sheet [2]. Heating to 70°C results in a conformational change, with a decrease in the negative peak and a shift to 205–206 nm, a decrease in the shoulder at 230 nm, and an isocircular dichroic point at about 213 nm. This suggests two defined conformations, one at high and one at low temperature, with a negligible concentration of intermediates. Determination of the dichroism at 228 nm (the wavelength of the greatest observed change) at 2 and 3°C intervals (fig.2b) shows that the change is monotonic over the temperature range. Calculation of a far-UV difference spectrum (not shown) by subtraction of the spectrum at 10°C from that at 70°C indicates that the change involves an increase in type B β-turns, as described [2].

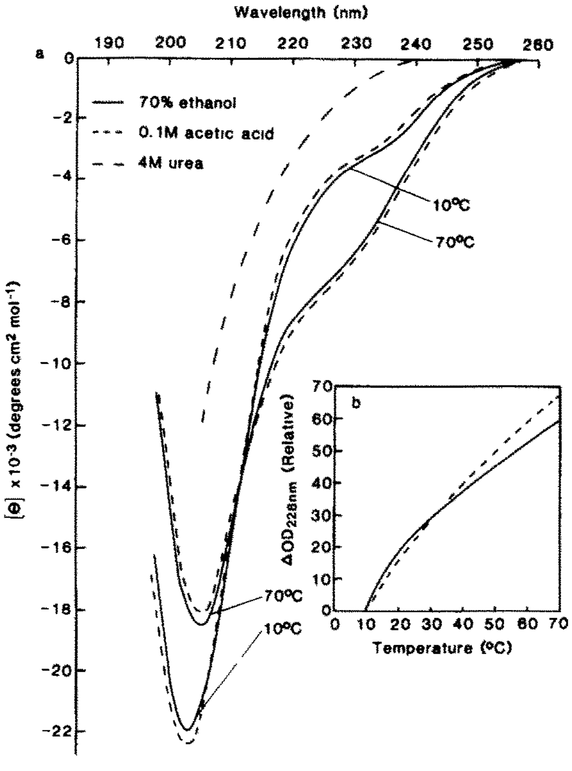

Fig.2. (a) The far-UV CD spectra of C hordein in 0.1 N acetic acid and 70% (v/v) ethanol at 10 and 70°C, and in 4 M urea at 20°C. (b) $[\theta]/[\theta]_0$ at 228 nm, measured at 2°C intervals between 10 and 40°C, and 3°C intervals between 40 and 70°C. The protein concentration was 1 mg/l·ml^{-1}.

The CD spectra in 0.1 M acetic acid are essentially similar to those in 70% ethanol, which is consistent with the viscometric results. The differences between the spectra in the two solvents, and the changes on heating, may again result from the different contributions of hydrogen bonding and hydrophobic interactions. These could affect the proportions of different β-turn types, and hence the CD spectra.

The molecular dimensions of C hordein are compatible with a helical conformation based on regularly repeated β-turns. The only such structure determined experimentally is the β-spiral, formed by the synthetic polypentapeptide of elastin [12]. This has 13.5 residues per turn, with a translation of 9.45 Å per turn. Assuming that C hordein consists of about 440 residues [1] with all except 18 present as repetitive penta- and octapeptides [2,3], a β-spiral similar to that reported for elastin would result in a molecule with dimensions of about 294 × 17 Å. This is within the range determined by viscometry (table 1).

We therefore conclude that C hordein is a rod-shaped molecule, with dimensions compatible with a helical secondary structure based on repetitive β-turns.

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