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Small-angle X-ray-scattering studies of the C hordeins of barley (*Hordeum vulgare*)

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Small angle X-ray scattering was used to study the solution conformation of the C hordeins of barley (*Hordeum vulgare*), a group of proteins whose primary structure consists predominantly of an octapeptide repeat motif. Measurements on the protein in 0.1 M-acetic acid at 25 °C are consistent with a model for the protein conformation of a stiff coil, the so-called 'worm-like' chain. The characteristic parameters (the Kuhn statistical segment length and the contour length) of the protein were calculated as 5.11 and 71.5 nm respectively.

INTRODUCTION

The C hordeins of barley (*Hordeum vulgare*), and the structurally related ω-gliadins of wheat (*Triticum aestivum*) and ω-secalins of rye (*Secale cereale*), comprise the sulphur-poor alcohol-soluble seed storage proteins (prolamins) of cereal grains. They account for some 5–15% of the total seed proteins and are characterized by an unusual amino acid composition: 40–50 mol% glutamine, 20–30 mol% proline and 7–9 mol% phenylalanine, with no cysteine and few or no methionine residues [1].

C hordein is a mixture of structurally similar proteins with identical *N*-terminal amino acid sequences [2,3]. Other sequence information comes from random chymotryptic peptides [4] and from cloned DNAs [5,6]. A C hordein pseudogene that encodes a protein of 327 residues (M_r 37800) has been isolated [7], and two ω-secalin genes encode proteins of 338 residues with M_r values of 39000 [8]. It is probable, therefore, that expressed C hordeins also have M_r values of about 40000. C hordeins appear to consist predominantly of an octapeptide repeat motif (consensus Pro-Gln-Gln-Pro-Phe-Pro-Gln-Gln) with short non-repetitive N- and C-terminal domains of twelve and six residues respectively.

Structure prediction, c.d. and i.r. studies of C hordein indicate the presence of β-reverse turns, with little or no α-helix or β-sheet. Studies of the whole protein and of a synthetic peptide corresponding to the repeat motif indicate the presence of type I/III β-reverse turns in equilibrium with a poly-L-proline II-like conformation, the position of equilibrium being influenced by solvent polarity and temperature [9]. In addition, preliminary viscometric studies were taken to suggest that the C hordein molecule is a semi-rigid rod with a diameter of about 2 nm (20 Å) [10].

The present paper describes small-angle X-ray-scattering (SAXS) studies which give more details of the molecular shape, dimensions and stiffness (flexibility) of C hordein.

MATERIALS AND METHODS

A total C hordein fraction was purified from barley (cv. Risø 56) as described previously, and purity was determined by *N*-terminal amino acid sequencing and SDS/PAGE [11].

SAXS experiments were performed at 25 °C, using quartz sample tubes and 0.1 M-acetic acid as the solvent. A Kratky SAXS camera (Anton Paar, Graz, Austria) was used for the measurements. X-rays were produced by a Cu tube driven by a Phillips PW1730 stabilized generator. Monochromatization of the Kα line was achieved using a tungsten filter and pulse-height discrimination. The X-ray wavelength was 0.154 nm. SAXS measurements spanned a broad range of scattering vectors (h = 0.122–5.31 nm^{-1}). Data were collected for the protein solution, solvent and empty cuvette by repeated sweeps through the angular range. Data were checked for time-dependent changes and then averaged. The protein-scattering curve was calculated as described by Müller [12]. The partial specific volume, ν, was calculated as 0.719 ml·g^{-1} [10].

Two concentrations of C hordein were used, namely 2.4 and 11.5 mg·ml^{-1}. The angular dependence of the scattering curve was the same at both concentrations, the curves scaling on top of each other. The scaling factor indicated a weak concentration-dependence.

The data collected at the higher concentration were used for further analysis because this gave a good signal-to-noise ratio over the entire angular range. The radius of gyration, R_g, was determined from the initial slope of a Guinier plot ($\log I$ versus h^2) where I is the scattering intensity and $h = (4\pi/\lambda)\sin\theta$, with 2θ the scattering angle and λ the X-ray wavelength. Parameters for the worm-like coil were obtained from a Holtzer [14] plot (hI versus h).

RESULTS AND DISCUSSION

SAXS was used to study the solution conformation of C hordein. In the limit of small scattering angles the SAXS of any non-interacting scatterer can be written in the form:

$$I(h) = \exp\left(\frac{-R_g^2 h^2}{3}\right)$$

The radius of gyration, R_g, can be obtained from the initial slope of the Guinier plot. Analysis of Fig. 1 gave an R_g value of 7.34 ± 0.08 nm. The interpretation of R_g in terms of the dimensions of the scattering particle requires an assumed model for particle shape. Considering a probable M_r of 40000, the measured R_g value for C hordein is high, suggesting a deviation from the simple 'random coil' or spherical globular model for the protein.

Schmidt *et al.* [13] have described a method, based on analysis first discussed by Holtzer [14], for determination of molecular

Abbreviation used: SAXS, small-angle X-ray scattering.
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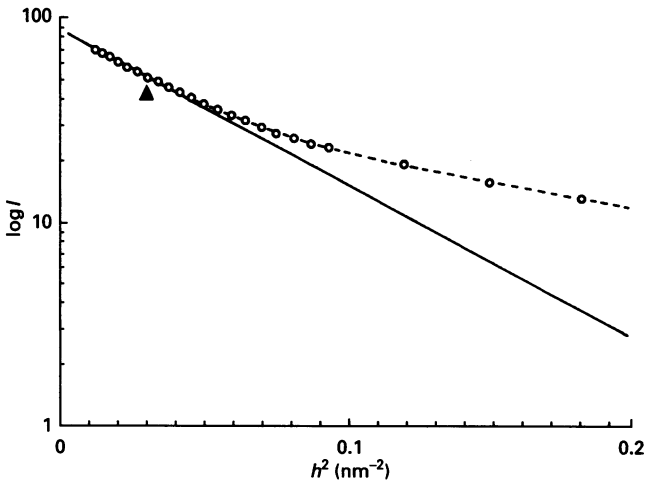

Fig. 1. Guinier plot (log I versus h^2) of X-ray-scattering data for C hordein in 0.1 M-acetic acid at 30 °C at 11.5 mg ml^{-1}

The arrow marks the range used for the $h \cdot R_g$ analysis and gives a value of approx. 1.3.

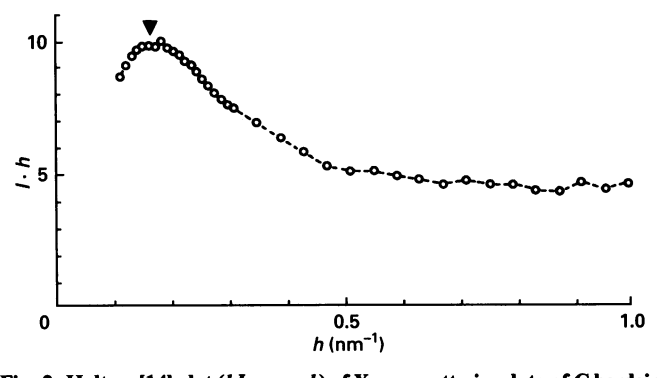

Fig. 2. Holtzer [14] plot (hI versus h) of X-ray-scattering data of C hordein in 0.1 M-acetic acid at 30 °C (after Schmidt *et al.* [13])

$h_{max.}$ is arrowed.

stiffness by plotting static scattering data in the form of a Holtzer plot (hI versus h). The shape of the Holtzer plot provides information on the shape and stiffness of the molecule [13]. For rigid rod-like molecules the plot of hI increases with increasing h, approaching a plateau value. For a stiff coil the plot of hI versus h initially increases, reaches a maximum value and then decreases to a plateau value. The higher the peak height relative to the plateau height the more flexible the coil. The Holtzer plot (Fig. 2) indicates that C hordein behaves as a stiff worm-like coil [14,15].

The stiffness of the molecule can be characterized by a Kuhn statistical segment length (l_k). If the contour length (the length along the molecule) of the molecule is L, then:

$$n_k l_k = L$$

where n_k is the number of Kuhn statistical segments in the coil. The position of the maximum in the Holtzer plot provides a measure of sample polydispersity:

$$U_{max.} = R_g \times h_{max.}$$

Theoretical calculations suggest a $U_{max.}$ value of 1.4 for monodisperse coils and 1.73 for polydisperse coils, assuming a most probable Schultz–Flory distribution [13]. For C hordein, $h_{max.} = 0.172 \pm 0.009$ nm^{-1} (from Fig. 2), giving $U_{max.} = 1.26 \pm 0.23$, suggesting that C hordein can be considered monodisperse. The ratio, R, of the height of the maximum to the plateau height (Fig. 2) gives a number related to the number of Kuhn statistical segment lengths (n_k). Using the value of $R = 2.4$, and a plot of R versus n_k for a monodisperse polymer [14,16], n_k was determined as 14. With the value for n_k it is possible to derive l_k using the relationship:

$$R_g^2 = \frac{n_k l_k^2}{6}\left\{1 - \frac{3}{2n_k} + \frac{3}{2n_k^2}[1 - (\exp - 2n_k)]\right\} \quad [17\text{–}19]$$

For $n_k = 14$ and $R_g = 7.34 \pm 0.08$ nm, l_k is calculated as 5.11 ± 0.05 nm and L, the contour length, as 71.5 ± 12 nm. The length per residue can be calculated, assuming the number of residues to be 338 (as determined for ω-secalins [8]), as 0.21 ± 0.04 nm per residue. These values are calculated from the R_g value at a finite concentration. The scaling of the data at 2.4 and 11.5 mg·ml^{-1} suggests a weak concentration-dependence which would result in a slightly smaller l_k value. However, the qualitative picture of C hordein as a stiff coil remains the most important conclusion from the present data.

Earlier studies on the hydrodynamic properties of C hordein in 0.1 M-acetic acid and aq. 70% (w/v) ethanol, using an Ostwald-type viscometer, were interpreted as implying an asymmetric rod shape for the molecule, with dimensions of 28.2 nm × 1.91 nm [10,20]. This was based on the assumption that the protein behaved as an asymmetric hydrodynamic particle rather than a random coil and that the M_r had a value of 52000 [10,20]. From hydrodynamic data, using intrinsic-viscosity values, determined by extrapolation of a concentration series to zero concentration, the Simha shape factor was calculated [21] (assuming an effective solvation of 0.2 g/g of protein) and used to calculate the axial ratios (a/b) of the prolate ellipsoid and the length and diameter of equivalent rod-shaped molecules [10,20]. The original data were recalculated using the M_r and ν value determined from the structurally related ω-secalin sequences [8]. Assuming a rod-shaped molecule the calculated dimensions for C hordein in 0.1 M-acetic acid at 30 °C were a length of 25.4 and diameter of 1.71 nm.

This interpretation of the viscosity data results from the high Simha value and the consequent assumption of an asymmetric rod-shaped particle. For the presently proposed stiff coils the effective volume occupied by the polymer will be large compared with the expected value for a globular protein. Thus, although the overall shape remains spherical, the increase in phase volume would manifest itself as an inflated Simha value, suggesting asymmetry. This can be tested by comparing the calculated R_g for the polymer coil obtained from the intrinsic viscosity with the measured value from SAXS data. Using the data in [10] we have an intrinsic viscosity [η] of 19.31 ml/g for C hordein in 0.1 M-acetic acid at 15.3 °C. The radius of gyration is given by:

$$R_g = \sqrt[3]{(3M_r[\eta]/10\pi N\xi^3)}$$

where N is Avogadro's number and ξ [22] is taken as 0.875 for ideal solvents and 0.775 for poor solvents. As reported in [10] this gave R_g values of 6.22 and 7.03 nm for ideal and non-ideal solvent conditions respectively. The calculated SAXS value is 7.34 ± 0.08 nm. Thus the X-ray and hydrodynamic data can be reconciled if the protein behaves as a coil. The SAXS data indicate local stiffness of the coil due to the unusual secondary structure.

C.d. and i.r. studies of the consensus repeat and whole protein have indicated an equilibrium between β-reverse turns and a poly-L-proline II-like structure; the position of the equilibrium being dependent on temperature and solvent polarity [9]. The viscometric data are consistent with this, the calculated dimensions varying with changes in solvent and temperature [10,20].

Matsushima *et al.* [23] have predicted and modelled poly-L-proline II and β-turn helices (termed pro-β helices) for a number of proteins containing repeated sequences rich in proline, phenylalanine or tyrosine, glycine, glutamine and serine. They developed a number of models for C hordein, using one and two β-turns per octapeptide repeat. All were elongated compared with other pro-β helices and had little instrastrand stabilization. Thus they could form hydrogen bonds with adjacent strands, both through the glutamine side chains and the amide groups of the protein backbone. The secondary structure of C hordein proposed by Matsushima *et al.* [23] is probably best described as containing short sections of poly-L-proline II and β-turn structures, although the length of the molecule calculated using their dimensions would be some 38 nm, that is, shorter than that determined. This might indicate a higher content of poly-L-proline II structure in C hordein than is indicated in the modelling work [23]. Matsushima *et al.* [23] suggested that these pro-β helices may represent a new class of secondary structure. The predicted pro-β helices are quite variable and flexible, which may relate to their function. The function of the sulphur-poor prolamins is only to act as a store of carbon and nitrogen for the developing seedling; in their case the structure may represent one that is easily packaged into compact protein bodies and/or digested by enzymes during germination.

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