Possible secondary structure in plant and yeast β -glucanase

E. Ann MacGREGOR* and G. Murray BALLANCE†‡

*Department of Chemistry, University of Manitoba, Winnipeg, Manitoba, Canada R3T 2N2, and †Department of Plant Science, University of Manitoba, Winnipeg, Manitoba, Canada R3T 2N2

Circular-dichroism spectra of a barley 1,3- β -glucanase were analysed by two methods. The combined results predict 36–40 % helix and 15–18 % β -structure in the protein. Prediction of secondary-structural features on the basis of amino acid sequence information yielded overall helix and β -structure contents of 37 % and 19 % respectively. Comparison of the predicted structural elements along the barley 1,3- β -glucanase with those of three related plant glucanases and a yeast glucanase suggest a close similarity in secondary structure among the five proteins. Consideration is given to the potential importance of certain amino acids which are conserved in these five glucanases.

INTRODUCTION

The endosperm cell walls of cereal grains are degraded during germination, thus allowing hydrolytic enzymes from the aleurone to come into contact with endosperm cell contents. In barley (*Hordeum vulgare*) both 1,3- β -glucanases (1,3- β -D-glucan glucanohydrolase, EC 3.2.1.39) and 1,3-1,4- β -glucanases (1,3-1,4- β -D-glucan 4-glucanohydrolase, EC 3.2.1.73) have been implicated in this process, and amino acid sequences of these enzymes have been determined [1,2]. Significant sequence similarity is evident between the two enzymes [1] and also with two other plant 1,3- β -glucanases, from *Nicotiana tabacum* and *Nicotiana plumbaginifolia* [3,4]. Although such similarity among protein sequences is usually indicative of relatedness in secondary and tertiary structure, nothing is known of the active conformations of these β -glucanases. Information on possible secondary structure in an enzyme can be useful to both X-ray crystallographers and investigators using mutagenesis to study structure–activity relationships.

We therefore present here an investigation of a barley 1,3- β -glucanase, using circular dichroism (c.d.) measurements and accepted methods of secondary structure prediction. The predictions are extended to a barley 1,3-1,4- β -glucanase and a *Saccharomyces cerevisiae* and two *Nicotiana* 1,3- β -glucanases.

MATERIALS AND METHODS

Barley endo-1,3- β -glucanase was purified from Havila barley as described previously [1]. C.d. measurements were carried out at 21 °C in a Jasco J-500A spectropolarimeter. The optical path was 0.5015 cm in a quartz cuvette, and spectra were scanned from 250 to 200 nm. Protein concentrations were in the range 0.025–0.13 mg/ml in sodium acetate/acetic acid buffer. The buffer was prepared by adjusting the pH of a 5 mM-sodium acetate solution to pH 5.5 with 5 mM-acetic acid solution. Protein concentration was determined by amino acid analysis on a Beckman 7300 high-performance amino acid analyser, with norleucine as internal standard. Each spectrum was corrected for the base-line contribution of the buffer system. The mean residue ellipticity $[\theta]$ (degrees $\cdot$ cm² $\cdot$ dmol^{−1}) was calculated from

$$[\theta] = \theta M_0 / 10lc$$

where θ is the measured ellipticity, M_0 is the mean molar residue weight = 105.4, calculated from the amino acid composition of

the protein [1], l is the optical path length in cm and c is the concentration in g/ml. The c.d. spectra were analysed to estimate protein secondary structure in accordance with Chang *et al.* [5] and Bolotina *et al.* [6]. Secondary-structure predictions were carried out by a combination of the methods of Garnier *et al.* [7] and Cid *et al.* [8], as described previously [9,10].

RESULTS AND DISCUSSION

C.d. spectrum of barley endo-1,3- β -glucanase

The c.d. spectrum of the barley 1,3- β -glucanase is shown in Fig. 1. The spectrum shown is an average of 30 spectra obtained from four enzyme solutions of different concentrations. The spectrum shows two negative bands with minima at approx. 210 nm and 222 nm, characteristic of a protein with an appreciable α -helix content. The overall shape of the spectrum is similar to that of many α/β proteins, i.e. proteins where there is some alternation of helix and β -strand along the polypeptide chain [11].

Different methods of analysis of c.d. spectra can give different estimates of secondary-structure content [12], and so more than one method is used here, in an attempt to obtain reliable values. An estimate of helix content from the mean residue ellipticity at 222 nm by the method of Chen *et al.* [13] gave 40 %. Analysis of the spectrum from 200 to 250 nm by the procedure of Chang *et al.* [5] (by using reference spectra from Table III of ref. [12]) yielded 40 % helix and 18 % β -structure, whereas the method of Bolotina *et al.* [6] gave 36 % helix and 15 % β -structure. From these results, the content of regular secondary structure is considered likely to be in the range 36–40 % helix and 15–18 % β -structure.

Prediction of regular secondary-structure content of plant and yeast 1,3- β -glucanases and a plant 1,3-1,4- β -glucanase

Although the procedure of Garnier *et al.* [7] is widely accepted for predicting the positions of regular secondary structure along a protein amino acid sequence, the predictive success of the method is generally around 56 % [14,15]. Combination with another procedure can improve the success rate [9,10], however, and so the method of Cid *et al.* [8] is also used here in an attempt to provide a reliable prediction. Predicted positions of regular secondary structure in the barley 1,3- β -glucanase are shown in Fig. 2. Probable error in the given positions is generally ± 2 . The

‡ To whom correspondence and requests for reprints should be addressed.

Fig. 1. C.d. spectrum of barley endo-1,3- β -glucanase

The c.d. spectrum (—) of barley 1,3- β -glucanase was determined and is shown as the variation of mean residue ellipticity $[\theta]$ with wavelength from 200 to 250 nm. Theoretical spectra, computed by the method of Chang *et al.* [5] (○) or Bolotina *et al.* [6] (●), are shown for comparison.

secondary structure predicted here amounts to 37% helix and 19% β -structure, in good agreement with the interpretation of the c.d. spectrum. Uncertainty exists, however, in assigning some elements of secondary structure as helix or β -strand, as indicated in Fig. 2. The helix content would rise to 42% and the β -strand content fall to 13% if all uncertain segments were assigned as helix. Conversely, these values would be 23% and 33% respectively if the segments were assigned as β -structure. These values, particularly the latter, appear less likely in the light of the interpretation of the c.d. measurements.

Using the same methods of structure prediction and the alignment of amino acid sequences shown, the positions of helices and β -strands in other β -glucanases [1–4,16] were determined (Fig. 2). The same structural elements are probably present in all four plant β -glucanases. The *S. cerevisiae* and both *Nicotiana* β -glucanases have segments which have no counterpart in the barley enzymes, but no attempt was made here to predict secondary structure in these segments.

The average helix length appears to be short (approx. nine residues) with glycine or proline residues within several helices. These helices, therefore, may be distorted, i.e. slightly bent. In some sections of the polypeptides, helices and β -strands appear to alternate along the chains (see Fig. 2), in agreement with conclusions drawn from the shape of the c.d. spectrum.

The secondary-structural elements given in Fig. 2 may differ in their importance for maintenance of the structural integrity of the enzymes. The yeast sequence lacks the structural elements corresponding to segments 18–36 and 181–202 of the barley 1,3- β -glucanase [16]. If the yeast sequence codes for an active enzyme, then the secondary structures in the two segments of the plant β -glucanases may not be necessary for the formation of the active conformation of the enzymes, or can be replaced by

B	IG---VCYGVIGNLPS-RSD	17
Nt	MAAITLLGLLLVASSIDIAGQSIG---VCYGLGNLNP-HWE	40
Np	ALQMAAILLG-LLV-SSTEIVGAQSVG---VCYGLGNLNP-ASQ	41
B4	IG---VCYGMSSANNLPA-AST	17
Sc	MRFSTTLATAATLFFASQVSAIGELAFNLGVK-NNDGCKST	43
B	VVQLYRSKGINRMRIYFADGQALSALRN-SGIGLILDIGN-DQLANIAAS	65
Nt	VIQLYKSRNIGRLRLYDPNHGALQALRG-SNIEVMLGLPNSD-VKHIA-S	87
Np	VVQLYKSNIRRMRLYDPNQALQALRG-SNIEVMLGVPNSD-LQNTAAN	89
B4	VVSMFKSNGIKSMRLYAPNQALQAVGG-TGINVVVGAPN-DVLSNLAAS	65
Sc	-----SD-----Y-----ETELQALKSYTSTVKVYAA-----SD-CNTLQNL	72
B	-TSNAASW-VQNNVRPPYPAVNIKYIAAGNEV-----QGGATQSLIPAMR	108
Nt	G-MEHARWVQKNVDFWPDVKIKYIAVGNEISPTGTSTYLSFLTPAMV	136
Np	-PSNANNW-VQRNVRNFWPAVKFRIYAVGNEVSPVTGTSLLRYLLPAMR	137
B4	-PAAAASW-VKSNIAQ-YPKVSFRYVCVGVNEV-----AGGATRNLYPAMK	107
Sc	GPAAEAGFTIFVG--VWPTDDSHYAAEKAAAL-----QTYLPKTK	112
B	NLNAALSAAG-----LGA-IKVSTSR	129
Nt	NIYKAIGEAG-----LGNNIKVSTSD	158
Np	NIRNAISSAG-----LQNNIKVSTSD	159
B4	NVHGALVAAG-----LGH-IKVTSVS	128
Sc	ESTVAGFLVGYSEALYRNDLTASQLSDKINDVRSVVADISDSOGK-SYSGK	161
B	FDEVANSFPSPAGVFN---AYMTDVARLLASTGAPLLANVPYFYAYRDN	176
Nt	MTLIGNSYPPSQGSFRNDARWFTDPIVGLRDRAPLLVNIYFYFYSYGN	208
Np	MTLIGNSFPSPSQGSFRNDVRSFIDPIIGFVRGINSPLLVNIYFYFYSYAGN	209
B4	QAILGVFSPSPAGSFTGEAAAFMGVQVQLARTNAPLMANIYPYLAWAYN	178
Sc	QVGTVDSSNVLVAGYNSAVIEASDFV-----MANAFSYWQQTMT	200
B	PGSISLNYATFQ-PGTTVSDQNNGLTYTSLFDAMVDVYAALEKAGAPAV	225
Nt	PGQISLPYSLFTAPNVVVQDGSRG---YRNLFDAMLDVYAALEERSGGASV	256
Np	PRDISLPYALFTAPNVVVQDGSGLG---YRNLFDAMLDVYAALESRAGGGST	257
B4	PSAMDHGYALFNASGTVVRDGAIG---YQNLFDFTTDAFYTAMGKHGSSV	226
Sc	QNAS-----YSFFDDIMQALQVIQSTKGSTDI	228
B	KVVVSESGWPSAGG-FAASA---GNARTYNOGLINHVGSGGTPKKREA-LE	270
Nt	GIVVSESGWPSAG-AFGATY---DNAATYLRNLIQHAKESPRKPGP-IE	301
Np	EIVVSESGWPSAG-AFAATT---NNAATYTKNLIQHVKRGSPPRPKNVIE	303
B4	KLVVSESGWPSGGG-TAATP---ANARFYNOHLINHVRGTRPHPGA-IE	271
Sc	TFVWGETGWPTDGTNFESSYPSVDNAQFQWKEGICSMRWG-----VN	271
B	TYIFAMFENQKTGDAT-----ERSFGLFNPDKSPAYNIQF	306
Nt	TYIFAMFDENKNPEL-----EKHGLFSPNKPQKYNINFGVSGGVWDSS	346
Np	TYLFAMFDENKNPEL-----EKHGLFSPNKPQKPLSFGFSDRYWDIS	348
B4	TYIFAMFENQKDSGV-----EQNWGLFYPNMQHVYPINF	305
Sc	VIVFEAFDEWDKPNSTSGTSDVEKHGVTSSDNLKYSLDCDFS	313
B	VETNAT-ASLVSEM	359
Nt	SENNATAASLISEM	362

Fig. 2. Amino acid sequence alignment and predicted secondary structures of β -glucanases

Amino acid residues are given in single-letter code, and numbering of residues from the N-terminus of the enzymes is shown. The enzymes are 1,3- β -glucanases from: B, barley [1]; Nt, *Nicotiana tabacum* [3]; Np, *Nicotiana plumbaginifolia* [4]; Sc, *Saccharomyces cerevisiae* [16]; and a 1,3-1,4- β -glucanase from barley, B4 [2]. Shaded areas show probable positions of secondary structure. H or β indicates helix or β -sheet, respectively, whilst H? and $\beta?$ indicate uncertainty in the assignment of the type of secondary structure and * denotes conserved acidic residues.

structures present in those yeast segments which are absent from the plant enzymes.

Conserved amino acids

Several amino acid residues are conserved in all four plant β -glucanases and the yeast enzyme. It is reasonable to assume that these play an important part in the structure or activity of the enzymes.

Carboxy groups have been implicated in the activity of several

1,3- β -glucanases [17–20], and here five such groups (equivalent to Asp-57 and -207 and Glu-231, -279 and -288 of the barley 1,3- β -glucanase) occur in all five enzymes. It has been proposed that sequences around amino acid residues essential for catalytic activity are more highly conserved, in related enzymes, than other sequences [21]. On this basis, the most likely candidates for involvement in catalytic activity are glutamic acid residues equivalent to 231 and 279 of barley 1,3- β -glucanase.

Histidine residues have also been considered important for β -glucanase activity [17,18,20,22,23]. Although no histidine residue is conserved in the plant and yeast sequences, a residue equivalent to His-257 of the barley 1,3- β -glucanase does occur in the four plant enzymes and hence may be involved at the active site of these enzymes.

Other conserved residues, particularly those with hydrophobic character, found in the plant and yeast enzymes are likely to be of structural importance. These would be amino acids equivalent to Ala-42, -113 and -247, Gly-2, -6, -118, -221, -233, -238 and -292, Ile-255, Leu-40, Phe-206, -274, -277 and -294, Pro-82, -105 and -235 and Val-229 of the barley 1,3- β -glucanase. Conserved residues that may have a structural role or may interact with the substrate at the active site include residues equivalent to Asn-10, -11, -166 and -246, Lys-123 and -282, Ser-127, Trp-234 and Tyr-33, -88, -170 and -302 of the barley 1,3- β -glucanase.

Although no deductions can yet be made about the tertiary structures of these β -glucanases, we believe the results presented here provide information which can help pinpoint segments and individual residues of the proteins, on which site-directed mutagenesis studies could be concentrated in order to gain greater understanding of the relationship between structure and function in these enzymes.

We thank Dr. W. G. Baldwin of the University of Manitoba for the computer program of the method of Garnier *et al.* [7] and Dr. C. C. Bigelow of the University of Manitoba for the use of the JASCO spectropolarimeter. We also thank Dr. R. Tkachuk of the Canadian Grain Commission for the amino acid analyses.

REFERENCES

1. Ballance, G. M. & Svendsen, I. (1988) *Carlsberg Res. Commun.* **53**, 411–419
2. Fincher, G. B., Lock, P. A., Morgan, M. M., Lingelbach, K., Wettenthal, R. E. H., Mercer, J. F. B., Brandt, A. & Thomsen, K. K. (1986) *Proc. Natl. Acad. Sci. U.S.A.* **83**, 2081–2085
3. Shinshi, H., Wenzler, H., Neuhaus, J.-M., Felix, G., Hofsteenge, J. & Meins, F., Jr. (1988) *Proc. Natl. Acad. Sci. U.S.A.* **85**, 5541–5545
4. DeLoose, M., Alliotte, T., Gheysen, G., Genetello, C., Gielen, J., Soetaert, P., Van Montagu, M. & Inzé, D. (1988) *Gene* **70**, 13–23
5. Chang, C. T., Wu, C.-S. C. & Yang, J. T. (1978) *Anal. Biochem.* **91**, 13–31
6. Bolotina, I. A., Chekhov, V. O., Lugauskas, V. Y. & Ptitsyn, O. B. (1980) *Mol. Biol. (Moscow)* **14**, 902–909; *Engl. transl.* (1981) **14**, 709–715
7. Garnier, J., Osguthorpe, D. J. & Robson, B. (1978) *J. Mol. Biol.* **120**, 97–120
8. Cid, H., Bunster, M., Arriagada, E. & Campos, M. (1982) *FEBS Lett.* **150**, 247–254
9. MacGregor, E. A. (1988) *J. Protein Chem.* **7**, 399–415
10. MacGregor, E. A. & Svensson, B. (1989) *Biochem. J.* **259**, 145–152
11. Manavalan, P. & Johnson, W. C. (1983) *Nature (London)* **305**, 831–832
12. Yang, J. T., Wu, C.-S. C. & Martinez, H. M. (1986) *Methods Enzymol.* **130**, 208–269
13. Chen, Y.-H., Yang, J. T. & Martinez, H. M. (1972) *Biochemistry* **11**, 4120–4131
14. Kabsch, W. & Sander, C. (1983) *FEBS Lett.* **155**, 179–182
15. Nishikawa, K. (1983) *Biochim. Biophys. Acta* **748**, 285–299
16. Klebl, F. & Tanner, W. (1989) *J. Bacteriol.* **171**, 6259–6264
17. Yamamoto, S., Mori, H. & Nagasaki, S. (1981) *Agric. Biol. Chem.* **45**, 2695–2703
18. Yamamoto, S., Miyagi, M. & Nagasaki, S. (1983) *Carbohydr. Res.* **114**, 137–145
19. Ohno, N., Hashimoto, Y. & Yadomae, T. (1986) *Carbohydr. Res.* **158**, 217–226
20. Ohno, N., Nono, I. & Yadomae, T. (1989) *Carbohydr. Res.* **194**, 261–271
21. Zvelebil, M. J. J. M. & Sternberg, M. J. E. (1988) *Protein Eng.* **2**, 127–138
22. Peterson, D. R. & Kirkwood, S. (1975) *Carbohydr. Res.* **41**, 273–283
23. Jeffcoat, R. & Kirkwood, S. (1987) *J. Biol. Chem.* **262**, 1088–1091

Received 29 June 1990; accepted 19 October 1990