Biochem. J. (1990) 270, 313–318 (Printed in Great Britain)

Conformational studies of peptides corresponding to the coeliac-activating regions of wheat α-gliadin

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The structures of four peptides corresponding to parts of the coeliac-activating protein A-gliadin were studied by structure prediction and c.d. spectroscopy. Three of the peptides corresponded to parts of the coeliac-activating *N*-terminal region (residues 3–55, 3–19 and 39–45) and contained two tetrapeptide motifs common to all coeliac-active regions (Pro-Ser-Gln-Gln and Gln-Gln-Gln-Pro). The Pro-Ser-Gln-Gln sequence was also present in the fourth peptide, on the basis of the *C*-terminal part of the molecule (211–217). These studies showed that β-reverse turns were the predominant structural feature in all peptides and were predominantly of type I/III in two of the *N*-terminal peptides and type II in the *C*-terminal peptide. These turns form when the peptide is dissolved in solvents of low dielectric constant (trifluoroethanol) and high dielectric constant (water and iso-osmotic saline), although their presence in the *N*-terminal peptides may be masked in the latter solvents due to equilibrium with a poly-L-proline II structure favoured at lower temperatures.

INTRODUCTION

Coeliac disease is a disease of humans that is characterized by injury to the small-intestinal mucosa, resulting in malabsorption. The disease occurs in genetically predisposed individuals after ingestion of the prolamin (alcohol-soluble protein) fractions of the grain of wheat, barley, rye and, rarely, of oats [1,2]. The mechanism by which the prolamins affect susceptible individuals is not known, despite numerous attempts at identification [3]. One hypothesis is that an abnormal immune antibody response follows ingestion of the proteins. However, the relevance of antibodies to prolamins in disease pathogenesis is uncertain, because affected individuals also have raised antibody titres against other food proteins, partly as a result of increased mucosal permeability [3]. Cell-mediated immune mechanisms are more likely to play a role in disease pathogenesis [1], but the way in which these processes injure the intestinal mucosa are not well understood. There is much interest in identifying the protein components that activate the disease and that are concerned with pathogenesis, and attention has focused on one group of proteins, the α-type gliadins, which are known to activate the disease in susceptible individuals.

Kasarda and co-workers [4] have made detailed studies of A-gliadin, a major α-gliadin subfraction, and peptides derived from it. Three CNBr-cleaved peptides spanning the A-gliadin molecule were tested for activity by organ culture of jejunal mucosa *in vitro* [5] and by murine T-cell proliferation [6]. In both systems the *N*-terminal peptide (1–127) was most active, the central peptide (128–246) less active and the *C*-terminal peptide (247–266) inactive. In addition, chymotryptic peptides corresponding to residues 1–30 and 31–55 were both active in the organ-culture system, whereas residues 56–68 were inactive. Wieser *et al.* [7,8] isolated coeliac-active peptides from an enzymic digest of whole gliadin and showed they corresponded to residues 3–24 and 25–55 of α-gliadin.

It was noted [5] that all coeliac-active peptides of A-gliadin have two tetrapeptide motifs in common, namely Pro-Ser-Gln-Gln and Gln-Gln-Gln-Pro. In addition the Pro-Ser-Gln-Gln motif is present in part of the A-gliadin sequence (residues 206–217) that is related to a sequence present in the E1B coat protein of human adenovirus Ad 12 [9]. It has been suggested that the viral protein may play a role in the pathogenesis of coeliac disease, possibly owing to immunological cross-reactivity between shared antigenic determinants [9].

In the present paper we report structure-prediction and c.d. studies of the conformations of one enzymically prepared peptide corresponding to residues 3–55 of A-gliadin and of three synthetic peptides (corresponding to residues 3–19, 39–45 and 211–217), two of which contain the tetrapeptide motifs common to all coeliac-active peptides. Our data indicate that, under physiological conditions, β-reverse turns are the predominant structural feature in residues 3–55, and that these are in equilibrium with an extended poly-L-proline II structure. It is possible that these β-reverse turns may play a major role in the pathogenesis in susceptible individuals.

MATERIALS AND METHODS

A peptide corresponding to residues 3–55 (peptide 1) of A-gliadin was prepared from an enzymic digest of total gliadin as described previously [7]. Unblocked linear peptides corresponding to residues 3–19 (peptide 2), 39–45 (peptide 3) and 211–217 (peptide 4) were synthesized commercially to a purity of 97%.

The c.d. spectra were recorded on a Jasco J600 spectrophotometer with a Jouan attachment for low-temperature measurements. The temperature was measured, by a thermocouple of a Comark electronic thermometer inserted directly into solution, to an accuracy of ±0.5 °C. The concentrations of peptides for c.d. spectroscopy were determined by weight. Corrections for peptide concentration due to solvent contraction at low temperatures were made with the data of Korver & Bosma [10]. Concentrations of peptide from 0.02 to 2.0 mg·ml^{-1} were used, no aggregation being observed in this concentration range. The spectra represent an average of at least two measurements and are expressed as mean residue $\Delta\epsilon$, the units for which are cm^2·mol^{-1} [11].

Abbreviation used: TFE, trifluoroethanol.

Structure predictions were made by the method of Chou & Fasman [12]. For β-turn analyses, a cut-off of 1.00×10^{-4} was used, where the average probability of a tetrapeptide adopting a β-turn conformation is 0.55×10^{-4}. Chou & Fasman [12] reported that 58% of β-turns are exactly located with this cut-off and that 78% are correctly localized within ±two residues.

RESULTS AND DISCUSSION

Conformations of the peptides

The A-gliadin molecule consists of an *N*-terminal domain of 95 residues, rich in proline and glutamine and based on short repeated sequences, and a longer (171 residue) *C*-terminal domain that is proline-poor and non-repetitive [13]. Peptide 1 corresponds to residues 3–55 of the *N*-terminal domain and also contains the sequences of peptides 2 and 3 (residues 3–19 and 39–45 respectively). Structure prediction of this peptide indicates a number of β-turns (Fig. 1) and one region of β-sheet. The peptide contains two each of the putative active tetrapeptide motifs Pro-Ser-Gln-Gln and Gln-Gln-Gln-Pro, three of which are involved in β-turn formation (Fig. 1). In addition, two β-turns involving parts of the tetrapeptide motifs are predicted for residues Gln-Pro-Phe-Pro (residues 35–38 and 47–50; labelled 'c' in Fig. 1), but these have probabilities (0.98×10^{-4}) just below the cut-off value used for turn prediction (1.00×10^{-4}). The sequence Gln-Pro-Phe-Pro has, however, been demonstrated to form β-turns in previous studies of a synthetic peptide and in proteins of related sequence [14]. Thus a number of overlapping β-turns, some involving the tetrapeptide motifs, are predicted to form in the *N*-terminal peptide, accounting for approx. 45% of the structure. Peptide 4, residues 211–217, is predicted to contain a single β-turn involving residues Arg-Pro-Ser-Gln (Fig. 1).

The conformations of the four peptides were also determined by c.d. spectroscopy. The far-u.v. (below 250 nm) spectrum arises principally from the absorption of the peptide bond and is indicative of the conformation of the peptide backbone. Spectra of the peptides were initially determined in trifluoroethanol (a solvent that promotes ordered hydrogen-bonded structures), water and iso-osmotic saline.

Peptide 1 showed a spectrum in trifluoroethanol (TFE) with negative maxima at about 205–206 and 221–223 mm (Fig. 2*a*). Although this type of spectrum is usually associated with α-helix [11], such a structure is unlikely to form, owing to the positioning and content of proline residues (28 mol %), which disrupt α-helical structures [12]. The spectrum is also compatible with a βI/III reverse-turn conformation, which has been demonstrated to exhibit α-helical-like c.d. spectra [15,16]. This conformation is more likely in view of the predicted structures. In water the spectrum showed a single negative maximum at about 201–202 nm, resembling that of the random-coil state [11]. A similar spectrum was observed in iso-osmotic saline (results not shown).

Peptide 2 showed a similar spectrum to peptide 1 in TFE, with negative maxima at about 203–204 and 223–224 nm (Fig. 2*b*), and in water a single negative maximum at about 199–200 nm (Fig. 2*b*). A similar spectrum to that in water was observed in iso-osmotic saline, with a negative maximum about 200 nm (spectrum not shown). As with peptide 1, the spectra in water and iso-osmotic saline were similar to that associated with the random-coil structure.

Peptide 3 in TFE showed a positive maximum at about 195 nm and negative maxima at about 208 and 225–226 nm (Fig. 2*c*); the spectrum did not show marked maxima or minima in water (Fig. 2*c*) or iso-osmotic saline (results not shown). The spectrum in TFE is not typical of any β-turn type [11] and most probably arises from the absorption of the tyrosine residue in the peptide; the absorption of the peptide in water is less intense. Similar types of spectra have been reported for poly-L-tyrosine [17]. Thus little information on the secondary structure can be obtained from these spectra.

Peptide 4 in TFE showed a spectrum with a positive maximum at about 215–217 nm, and a negative maximum below 200 nm, indicative of a type II β-turn (Fig. 2*d*) [11,15]. In water the spectrum showed a loss of intensity of the 215–217 nm band and an increase in intensity of the 200 nm band, indicating a reduction in the content of β-turns. The positive maximum observed in water suggests that at least a proportion of the peptide was in the β-turn conformation. A similar spectrum was observed in iso-osmotic saline (results not shown).

Variable-solvent and -temperature c.d. studies

The initial studies indicated that the peptides contained type I/III (peptides 1 and 2) and type II (peptide 4) β-turns when dissolved in TFE (a solvent of low dielectric constant), but peptides 1 and 2 gave random coil-like spectra when dissolved in water and iso-osmotic saline (solvents of high dielectric constant). The transition between these conformations for peptide 1 was studied with TFE/water (1:1, v/v) (Fig. 2*a*). The spectra show an isosbestic point at about 215 nm, indicating an equilibrium between two conformational states. The c.d. spectrum of peptide 1 in iso-osmotic saline was independent of pH between pH 2.5 and 10.5 (spectra not shown), showing similar spectra to that in water. Considering the lack of charged residues, apart from a glutamic acid residue at position 20 of peptide 1, the result was not unexpected.

The solution-state conformational analysis of linear peptides at room temperature is limited, as they are usually flexible and undergo conformational interconversion. More precise information concerning conformation can be obtained from variable-temperature studies, particularly under cryogenic conditions, where low temperature induces freezing to a more limited range

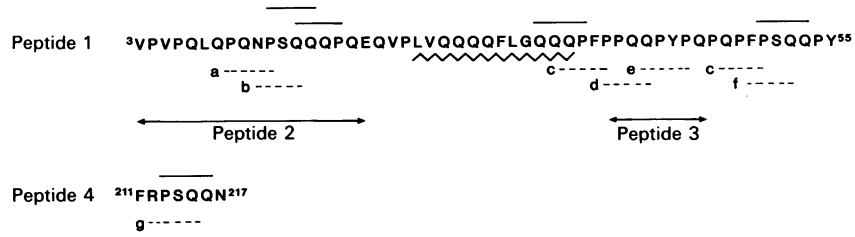

Fig. 1. Sequences and structure prediction of peptides 1–4

Tetrapeptide sequences common to all coeliac active peptides (——) and predicted β-turns (----). Probabilities of turns ($\times 10^4$): (a) 1.29, (b) 5.94, (c) 0.98, (d) 1.11, (e) 1.73, (f) 2.17 and (g) 2.58, where the average probability of a turn occurring is 0.55. ⁓⁓, β-sheet.

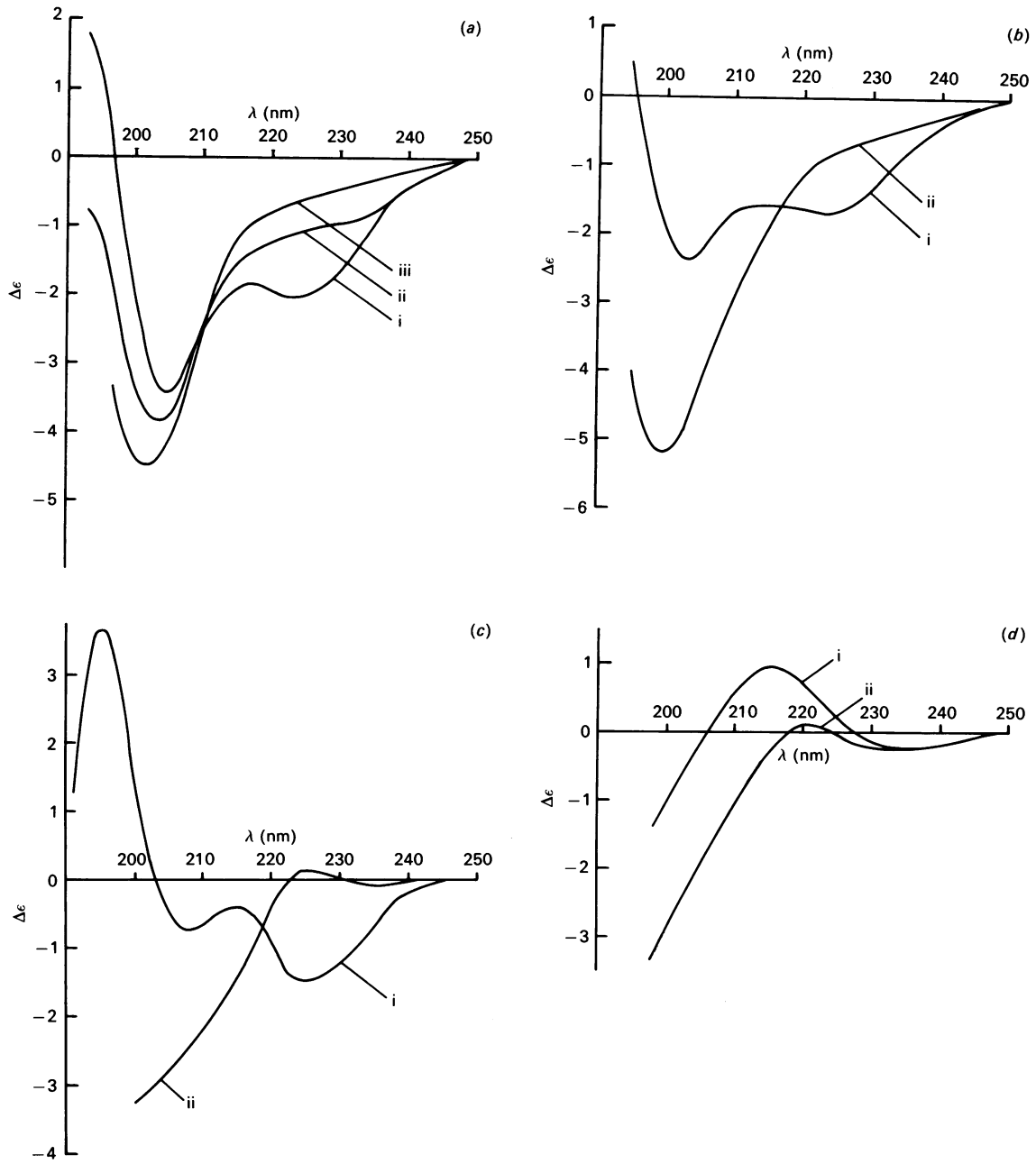

Fig. 2. C.d. spectra of the peptides in TFE and water

(*a*) Peptide 1: i, TFE; ii, TFE/water, 1:1 (v/v); iii, water. Peptides 2(*b*), 3(*c*) and 4(*d*): i, TFE; ii, water. The peptide concentration was 0.2 mg·ml^{-1} and the temperature 20 °C.

of conformations (often the most stable). The conformations of the peptides were therefore studied as a function of temperature in the cryogenic solvent system ethanediol/water (2:1, v/v), which has been used as an analogue of water [14].

The spectrum of peptide 1 in ethanediol/water (2:1, v/v) at 20 °C was similar to that in TFE/water (1:1, v/v) (Fig. 2*a*), indicating that a proportion of the peptide was in the β-turn conformation. On cooling the solution the spectrum changed markedly, the negative maximum at about 199–200 nm increasing in intensity and shifting to a lower wavelength (<200 nm at −100 °C). A positive maximum also developed at about 223–224 nm (Fig. 3*a*). The spectra exhibited an isosbestic point at about 205–206 nm, indicating an equilibrium between two conformational states. The spectrum at −100 °C does not resemble those associated with α-helix, β-sheet or random coil [11], or any theoretical or observed spectrum reported for a β-reverse-turn type [15,17]. It does, however, resemble the spectrum associated with the extended left-handed helical structure of poly-L-proline II, which has a negative maximum at about 200 nm and a positive maximum at about 223–225 nm [11,18,19]. To determine if hydrogen-bonded structures, such as β-turns, contribute significantly to the spectrum at −100 °C, the cooling was repeated in the presence of 4 M-urea to disrupt hydrogen-bonding (poly-L-proline II does not contain intramolecular hydrogen bonds). Similar spectra were observed in the 205–250 nm range (not shown), indicating that β-reverse turns were not contributing significantly to the spectrum at −100 °C. This does not, however, exclude the possibility of a contribution from open reverse turns, that is, β-turns which do not contain intramolecular hydrogen bonds.

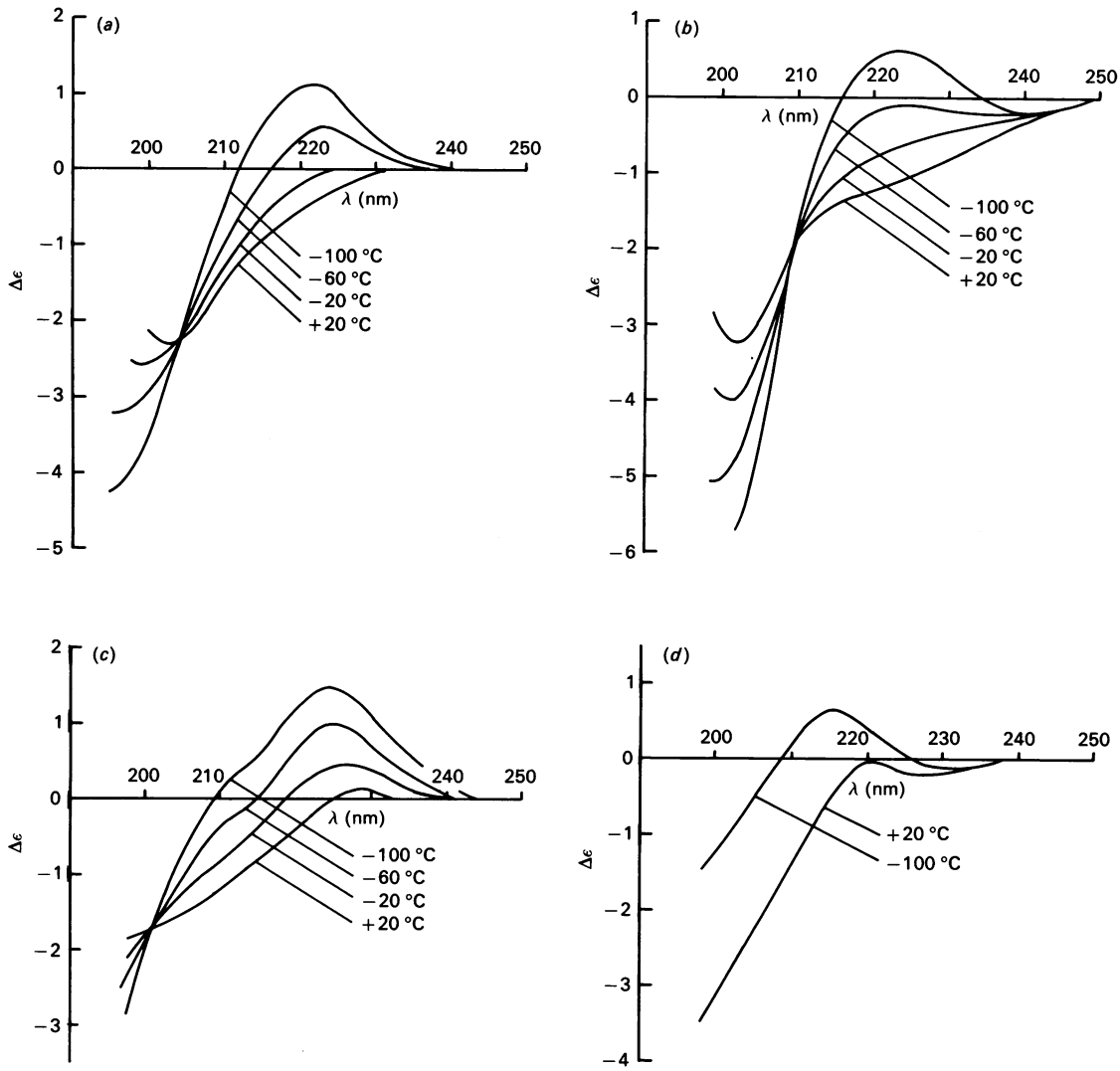

Fig. 3. Low-temperature c.d. spectra of the peptides in ethanediol/water (2:1 v/v)

(*a*) Peptide 1; (*b*) peptide 2; (*c*) peptide 3; and (*d*) peptide 4. The peptide concentration was 0.2 mg·ml^{-1}.

Peptide 2 showed similar spectra to peptide 1 at 20 °C and on cooling (Fig. 3*b*), the negative maximum at about 202–203 nm at 20 °C increasing in intensity and shifting to a lower wavelength (< 200 nm) at −102 °C. The negative maximum at about 224 nm decreased in intensity to give a positive maximum at about 224–225 nm (Fig. 3*b*). The spectra exhibited an isosbestic point about 211–212 nm, indicating an equilibrium between two conformational states. The addition of 4 M-urea did not affect the spectrum at −102 °C (results not shown).

The spectrum of peptide 3 in ethanediol/water at 20 °C was similar to that in water (Fig. 2*c*). On cooling to −100 °C the spectrum showed a negative maximum at about < 230 nm and a positive maximum at about 224–225 nm (Fig. 3*c*). The low-temperature spectra were similar to those of peptides 1 and 2.

Peptide 4 in ethanediol/water at 20 °C showed a spectrum similar to that in water (Fig. 2*d*). On cooling to −100 °C the spectrum showed increases in intensity of the positive and negative maxima at about 216–217 nm and < 200 nm respectively (Fig. 3*d*). The spectrum at low temperatures was consistent with the formation of β-turns, not poly-L-proline II structure.

In ethanediol/water (2:1, v/v), peptides 1 and 2 would appear to be in equilibrium between a predominantly β-turn-rich conformation at higher temperatures and a poly-L-proline II structure at lower temperature. The usual requirements for a poly-L-proline II structure to form are repeated blocks of proline residues or proline at every third position of the polypeptide chain [20]. The position of the proline residues does not entirely satisfy these requirements; however, the restricted conformational freedom of the proline residues at low temperature may favour the poly-L-proline II conformation. The conformation of peptide 3 in TFE and water cannot be determined, owing to the intense tyrosine absorption; however, at low temperatures it appears to adopt a poly-L-proline II-like structure, the contribution of the tyrosine residue to which cannot be determined. Peptide 4 appears to be predominantly a type II β-turn over a range of solvent and temperature conditions and shows no evidence of poly-L-proline II-like structure.

Modelling of spectra

The random-coil-like c.d. spectra observed in iso-osmotic saline and water for peptides 1 and 2 may not represent true random-coil conformations, but averages of the two major conformations observed under different conditions: βI/III reverse turns and the left-handed extended poly-L-proline II helix. This view is supported by the transitions between the two conformations that occur as a function of solvent and tem-

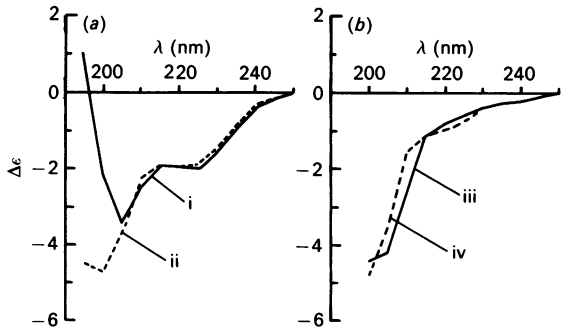

Fig. 4. Calculated and experimental c.d. spectra of peptide 1 in (*a*) TFE and (*b*) water

(*a*): i, experimental; ii, calculated by assuming 50% βI/III reverse turn and 50% poly-L-proline II. (*b*): iii, experimental; iv, calculated for 30% βI/III reverse turn and 70% poly-L-proline II.

perature and the presence of isosbestic points. As the spectra of peptides 1 and 2 derive from β-reverse turns and poly-L-proline II structure, it is possible, using reference spectra for these two conformations, to model the experimentally determined spectra. Spectra for peptide 1 in TFE and iso-osmotic saline were modelled on the spectrum of a cyclic hexapeptide [21], which has been demonstrated to form a βI/III reverse turn, and of poly-L-lysine at low temperature, where it adopts the poly-L-proline II structure [22]. Poly-L-lysine is considered to be a closer model for peptides in the extended left-handed poly-L-proline II-like conformation than poly-L-proline, owing to tertiary nitrogen in the backbone of poly-L-proline [22]. The structure has been characterized by vibrational c.d. in poly-L-lysine and a number of oligopeptides [23–25].

In TFE at 20 °C, a calculated spectrum consisting of 50% βI/III reverse turn and 50% poly-L-proline II was in good agreement with the observed spectrum (Fig. 4*a*) and with the predicted content of β-reverse turns (see above). In water at 20 °C the content of β-reverse turns is reduced and a calculated spectrum with 30% β-reverse turns and 70% poly-L-proline II shows good agreement (Fig. 4*b*). The results of a similar analysis for peptide 2 in TFE gave 40% β-reverse turn and 60% poly-L-proline II, and in water and iso-osmotic saline about 30% β-reverse turns and 70% poly-L-proline II (results not shown). The calculated and experimental spectra are in good agreement between 205 and 250 nm, but the fit is less good below 205 nm.

General discussion

β-Turns in coeliac-active peptides. The structure-prediction results indicate that β-reverse turns are the major secondary structure in all four peptides and involve three of the four putative active tetrapeptide motifs (Pro-Ser-Gln-Gln and Gln-Gln-Gln-Pro) that are present. These results are supported by the c.d. spectra, which indicate that β-turns are present in at least three of the peptides when dissolved in TFE, although their presence may be masked in peptides 1 and 2 when dissolved in water and iso-osmotic saline, owing to an equilibrium with poly-L-proline II structure. This results in random-coil-like spectra. Thus β-turns appear to be the major structural feature present in all peptides when dissolved under physiological conditions. Although β-sheet is also predicted for peptide 1, this structure is not present in peptides 2, 3 or 4. In addition, chymotryptic peptides corresponding to residues 1–30 and 31–55 are also coeliac-active [5,7,8], although cleavage between residues 30 and 31 would cut the region of predicted β-sheet. It is therefore unlikely that this conformation has any direct role in coeliac activity. It is not possible to determine whether the overlapping β-turns predicted for peptides 1 and 2 and elsewhere in the α-gliadin protein [26] actually form using c.d. or i.r. spectroscopy [26,27], but X-ray crystallography and n.m.r. spectroscopy have shown that overlapping β-turns do form in other proteins [28] and in synthetic peptides [29]. The latter study suggested that the resulting double-bend structure could provide an important site for recognition.

β-Turns in coeliac disease. Two features of the structure and properties of β-turns may be relevant to the pathogenesis of coeliac disease. Firstly, they are usually located at the surface of proteins, owing to their polar nature. As such they may be predicted to function as antigenic sites, and adjacent and overlapping β-turns have been shown to form compact loops which extend from the surface of proteins such as staphylococcal nuclease and to enhance their interactions with antibodies [30]. A similar surface location could facilitate interaction either with the mucosal surface or with antigen-presenting cells within the pamina propria of sensitized individuals. Secondly, β-turns usually form in short synthetic peptides (unlike regions of α-helix and β-sheet), so that it is possible to raise antibodies against such peptides that are reactive with conformational motifs present in the intact protein. This property may also be important in coeliac disease, as the gliadin is likely to be extensively digested within the lumen [31] before reaching the mucosal site where pathogenic mechanisms are triggered.

The studies reported here indicate that the coeliac-active peptides might adopt β-turn-rich conformations, as they interact with the luminal surface membrane of the intestine. This is because the dielectric constant at a membrane interface is reduced compared with that of the bulk phase, owing to the presence of structured water associated with charged lipid head-groups and counter-ions [32]. Values for the dielectric constant at the interfacial region vary and depend on the experimental method or method of calculation, although values of about 8–20 (cf. that for ethanol, 24) are often quoted [33,34]. The dielectric constant gradually increases through the Gouy–Chapman layer to a bulk-phase value of about 80, and this region can extend up to 75Å under physiological conditions [35]; hence these peptides could largely be accommodated within this layer. Thus it is possible that the reduced dielectric constant in the immediate environment of a membrane surface favours the adoption of β-turn-rich conformations by these small peptides, similar to those adopted in TFE.

Although β-turns may be involved in the activation of coeliac disease, they clearly do not provide a complete explanation. This is because β-turns are present in non-activating parts of the α-gliadin molecule, as well as in many other proteins. It is most likely, therefore, that the recognition events that elicit a pathological response in gluten-sensitive individuals involves a combination of specific sequence motifs and β-turns that must be recognized in the context of particular major histocompatibility complex ('MHC') class 2 specificities and T-cell receptor expression.

A.S.T. thanks Dr. A. F. Drake, Birkbeck College, University of London, for the use of c.d. facilities and useful discussion.

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Received 21 March 1990; accepted 9 April 1990