PURIFICATION AND PARTIAL SEQUENCE OF AN α-gLIADIN

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Coeliac disease (gluten enteropathy) is associated with a damaged gut mucosa displaying degraded villi and poor absorption of nutrients. The major symptoms are emaciation, swollen abdomen, poor growth, vomiting, diarrhoea and steatorrhoea [1]. The condition affects 1 in 1850 individuals in the UK although the world-wide incidence is variable and depends upon the dietary use of wheat and similar cereals. Coeliac disease is caused by an intolerance to the gluten content of cereal flours [1].

Gluten contains approximately equal portions of glutenin and gliadin proteins; only gliadins are pathogenic to susceptible individuals. Starch gel electrophoresis (SGE) separates gliadins into α-, β-, ω- and γ-fractions, in order of decreasing electrophoretic mobility [2]. More recently, gliadins have been classified according to their sizes, composition and amino terminal sequences [3]. The predominant groups in this classification are the sulphur-rich αβ- and γ-gliadins and the sulphur-poor α-gliadins. The α-gliadin fraction, comprising up to about 50 distinct polypeptides, is the most pathogenic. We recently reported the preparation of discrete α-gliadins using RP-HPLC [4] as a preliminary to investigating their structure. The utility of these methods has recently been confirmed [5].

Primary structures have been reported for some gliadins, mainly of the first 25 amino terminal residues [6, 7], although several sequences have been established for α-, β- and γ-gliadins from cDNA studies [8-13]. An analytical sequence, that by Edman degradation, has also been reported for the so-called A-gliadin fraction [9]. This extract consists of an aggregated mixture of gliadins obtained by precipitation, at specific values of pH and ionic strength, from commercial gluten and has often been used in structural studies because of the ease with which it can be prepared. The reported sequences of the α-gliadins can be arranged into six domains: an amino terminal repeating sequence (domain I); a polyglutamine section (II); a unique sequence (III); a second polyglutamine section (IV); a second unique sequence (V) and a carboxy terminal domain (VI).

This communication reports the isolation and partial sequence of an α-gliadin. The purity of the preparation was determined by matrix assisted laser desorption ionisation-time of flight-mass spectrometry (MALDI-TOF-MS), capillary electrophoresis (CE) and UV absorption spectrophotometry. All materials and equipment were purchased from reputable suppliers. Ion exchange and reverse phase chromatographies, SGE and MALDI-TOF-MS were all performed as described previously [4]. The CE was performed using an ISCO model 3850 capillary electrophorograph with a 75 μm internal diameter anionexchanger column in 4 mmol dm⁻³ sodium borate buffer, pH 3.5 at 5 kV for 10 s and then at 20 kV and 6 μA for approximately 6 minutes. Migration was monitored at 280 nm. The partial amino acid sequence was determined by automatic Edman degradation.

The repeated application of ion pairing RP-HPLC separated four discrete α-gliadins of M, 31 114.7, 31 156.0, 31 258.8 and 31 197.6 as determined by MALDI-TOF-MS. These values therefore represent a refinement of the 32-40 000 range previously reported [references cited in 14]. The sequence reported for the A-gliadin fraction has a similar M, of about 30 800 when calculated from its reported sequence [9].

The α-gliadin of M, 31 156 (Fig 1(a)) was isolated in the greatest amount and chosen for further study. Its purity was confirmed by CE (Fig 1(b)) and UV absorbance (results not presented).

Fig 1 (a) MALDI-TOF-MS and (b) capillary electrophoretogram of the α-gliadin preparation.

The primary structure of the preparation was partially determined. This sequence consisted of the first 30 amino terminal residues and a short section of internal residues. Both sections were identified unambiguously and are listed in Table 1. The amino terminal sequence is identical to that reported for the aggregated A-fraction [9] and to sequences predicted from cDNA molecules of αβ-gliadins, but differs from that predicted for some γ-gliadins [8-13]. The internal sequence is also homologous to those of the cDNA derived and A-fraction studies. This report of a direct sequence, albeit partial, of a known discrete α-gliadin molecule confirms the close sequence homologies of the α-gliadins which other workers have reported.

References