PERIODICA POLYTECHNICA SER. CHEM. ENG. VOL. 46, NO. 1–2, PP. 51–58 (2002)

APPLICABILITY OF CAPILLARY ELECTROPHORESIS IN PROTEIN SEPARATIONS WITH REGARD TO WHEAT AND WHEAT ANALOGOUS PROTEINS

Éva SCHOLZ*, Katalin GANZLER**, Szilveszter GERGELY* and András SALGÓ*

*Department of Biochemistry and Food Technology
Budapest University of Technology and Economics
H–1521 Budapest, Hungary
Tel.: +36 1 463 3857, Fax.: +36 1 463 3855
e-mail: scholz@mail.bme.hu
**Gedeon Richter LTD. Formulation R&D
H–1103 Budapest, Gyömrői út 19-21., Hungary

Received: March 30, 2003

Abstract

High-performance capillary electrophoresis (HPCE), among other analytical techniques (e.g. acid polyacrylamide gel electrophoresis, sodium dodecyl sulphate polyacrylamide gel electrophoresis and reversed-phase high-performance liquid chromatography) [1]–[4] is more and more widely used also in the field of separation of cereal proteins [5]–[18]. HPCE is versatile, easy to automate, does not need toxic reagents or long analysis time, but requires only small sample size, small amount of buffer and provides high-resolution separations. So this analytical technique is particularly applicable for studying the fine structure of the composition of wheat proteins.

Capillary electrophoresis has been used at our department for the determination of the fine structure of different wheat protein fractions [19]. Differences between the various wheat cultivars and changes during grain maturation process have also been studied using a home-built capillary electrophoretic system [20]. The same system has been used to investigate the electrophoretic properties of a gliadin analogous protein (BM180 – a basement membrane protein with a potential autoantigen role) [21].

The more strictly controllable nature of an automated system (especially the temperature control of the capillary) allows the use of higher voltages, so separation time decreases and resolution increases. Purchasing an automated capillary electrophoretic system we gained an opportunity to compare the two electrophoretic systems also in the field of wheat protein analyses.

The aim of present work was to give an impression of the work done by capillary electrophoresis at our department.

Keywords: capillary electrophoresis, capillary electrophoretic systems, wheat proteins, gliadins, maturation process.

1. Introduction

No one would question the role of cereal grains in human nutrition and animal feeding. Wheat is especially important among cereals. The quality of wheat and flour, and thus the quality of the cereal products are mainly dependent on the protein composition. Especially the gluten protein fraction (gliadins and glutenins) determine, due to their unique viscoelastic properties, the breadmaking quality of

wheat flour [22]. Studying the fine structure of these protein fractions is of service to cereal and food scientists.

Wheat proteins are difficult to characterise because of their complexity and interactions. They are usually classified according to their solubility. The fractions can be obtained with the sequential Osborne extraction procedure [23]. The protein composition of the gliadin fractions also changes during the maturation process and varies from cultivar to cultivar. So monitoring the fine structure of gliadin fractions can provide information regarding the wheat maturation process and also varietal identification.

Characterisation of wheat proteins by HPCE is usually performed in capillary zone electrophoresis (CZE) or sodium-dodecyl-sulphate capillary gel electrophoresis (SDS-CGE) modes. In CZE the use of a low pH phosphate buffer containing hydroxypropyl-methyl-cellulose (HPMC) as polymeric additive [6], [8], [9], [12]–[14] and the use of isoelectric buffers (e.g. iminodiacetic acid) [11], [12], [17] are most widespread. In SDS-CGE mainly polyacrylamide or dextran gel is the sieving matrix [5], [15], [21].

For studying of wheat proteins during maturation process and for wheat varietal identification we used a capillary zone electrophoretic method [19], [20], for monitoring the purification process of BM180 we used sodium dodecyl sulphate-capillary gel electrophoresis [21].

The aim of present paper was to summarise some results achieved by capillary electrophoresis in the field of wheat and wheat analogous protein analysis with special emphasis on comparison of the manual and automated capillary electrophoretic systems.

2. Materials and Methods

2.1. Chemicals

All of the chemicals used in this study were of analytical grade and were purchased from Fluka Chemie (Buchs, Switzerland) unless mentioned otherwise. For the preparation of the electrolytes double distilled Milli-Q water (Waters, Milford, MA, USA) was used. Acetonitrile (ACN), 1-propanol and ethanol were of HPLC grade and were purchased from Chemolab (Budapest, Hungary). Hydroxypropyl-methyl-cellulose (HPMC) and 2-mercapto ethanol were from Sigma (St.Louis, USA).

2.2. Wheat Samples

The Hungarian winter wheat cultivar Martonvásári 23 was obtained from the Agricultural Research Institute of the Hungarian Academy of Sciences in Martonvásár, Hungary. In the field test shoot samples were collected 12, 15, 23, 29, 33, 38, 43, 47 and 53 days after anthesis (DAA). Seeds were immediately prepared from shoot

and were stored at −20 °C until analysis. Wheat meals were obtained with manual grinding of the grain in a mortar and pestle.

2.3. Osborne Fractionation

Gliadins were isolated after the removal of albumins and globulins following a variation of the sequential Osborne procedure [23] as described previously [19].

The maturation process could be well characterised with the fractions from the first extractions, because these contained the majority of proteins [8].

2.4. Capillary Electrophoretic Instruments and Conditions

2.4.1. Home-Built Capillary Electrophoretic System

This system consisted of a CZE 1000 PN 30 power supply (SPELLMAN High Voltage Corporation, Plainview, NY, USA), a Spectra 100 UV/VIS detector (Thermo-Separation Products, San Jose, California, USA) and a DTK Personal Computer (Parity Ltd. Budapest, Hungary) equipped with an analogue to digital converter board (Data Translations, Framingham, MA, USA) and a data acquisition and analysis software (Caesar) (Analytical Devices Inc. Alameda, CA, USA). Fused-silica capillaries (Composite Metal Services Ltd., Worcs., GB) with the inner diameter of 50 μm were used.

All the analyses were performed at ambient temperature; the capillaries were cooled using a laboratory fan. Separations were carried out in uncoated fused silica capillaries with a length of 40 cm (20 cm separation distance). Separation voltage was 14.3 kV. Samples were detected by UV absorbance at 214 nm. The separation buffer was 100 mM phosphoric acid/β-Ala (pH 2.5) containing 1M urea, 0.05% hydroxypropyl-methyl-cellulose (HPMC) with viscosity of aqueous solution at 20 °C: $\sim$ 4.000 cP and 20% acetonitrile (ACN) and it was filtered through 0.45 μm filters before analyses. Capillary was conditioned using 1N NaOH, double distilled water, 0.1N HCl, double distilled water and the separation buffer between runs. Injection was carried out electrokinetically and the required time was 4 seconds.

2.4.2. Beckman P/ACE MDQ Capillary Electrophoretic System

The Beckman P/ACE MDQ system (Beckman Coulter, Inc., Fullerton, California, USA) includes a selectable-wavelength UV/VIS (200, 214, 254 and 280 nm filters installed) detector, UV source optics, temperature-controlled sample storage module (5 to 60 °C), autosampler and P/ACE MDQ 32 Karat Software configured on an IBM personal computer. The automated, programmable system is capable of a rinse pressure of −5 to +100 psi and an injection pressure of −5 to +25 psi. Constant

capillary cartridge temperature (15 to 60 °C) is maintained by recirculating liquid coolant. Fused-silica capillaries (Composite Metal Services Ltd., Worcs., GB) with the inner diameter of 50 μm were used.

During the wheat maturation analyses the capillary cartridge temperature was 40 °C; samples were kept at 6 °C. Separations were carried out in uncoated fused silica capillaries with a length of 31.2 cm (21 cm separation distance). Separation voltage was 20 kV. Samples were detected by UV absorbance at 214 nm. The separation buffer and the capillary cleaning procedure was the same as with the manual CE system. Injection was carried out with a pressure of 0.4 psi for 4 seconds.

3. Results and Discussion

Gliadins were analysed in capillary zone electrophoretic mode, where proteins are separated according to their charge densities. In the early phase of maturation no gliadins were observed. The quantity of gliadins increased rapidly after the 33*rd* day after anthesis (DAA) for the variety Martonvásári 23 (*Fig. 1*). The number and intensity of detected peaks changed intensively during maturation but a characteristic peak pattern developed as a function of maturation time. In the phase of late maturation the gliadin pattern remained mainly unchanged and only peak intensities varied. The gliadin patterns and the starting point of gliadin formation were different for other cultivars [20]. (Data not shown).

Using an automated CE system with capillary thermostating higher voltage could be used on shorter capillary length, the electric field almost doubled (641 V/cm instead of 357.5 V/cm), even though the observed current doubled, too. Raising the electric field reduced the separation time from 30 minutes to 6 minutes (*Fig.2*). Comparing the electropherograms of the same wheat protein fraction (gliadin fraction of Martonvásári 23 cultivar 53 days after anthesis) gained with the two different capillary electrophoretic systems (*Fig. 2*), it can be seen that with the manual electrophoretic system a few more peaks could be detected, but the resolution is slightly better with the automated one (and can be improved by decreasing the voltage and/or temperature, albeit at the expense of a little longer separation time). The radical decrease in separation time (6 minutes instead of 30 minutes) is an obvious advantage during wheat varietal identification. The retention time reproducibility is better for the automated system probably mainly because of the strict temperature control. With further optimisation of the separation method done with the automated CE system, resolution and reproducibility can be even more improved. Studying the maturation process of wheat gliadins with the manual electrophoretic system, uncertain pattern changes were observed in the later phase of maturation (*Fig.1*). It could not be determined whether the reason is methodological (the home-built capillary electrophoretic system does not have sufficient capillary cooling) or physiological, without the use of an automated system with sufficient capillary temperature control. With the automated system these uncertain pattern changes were not observed

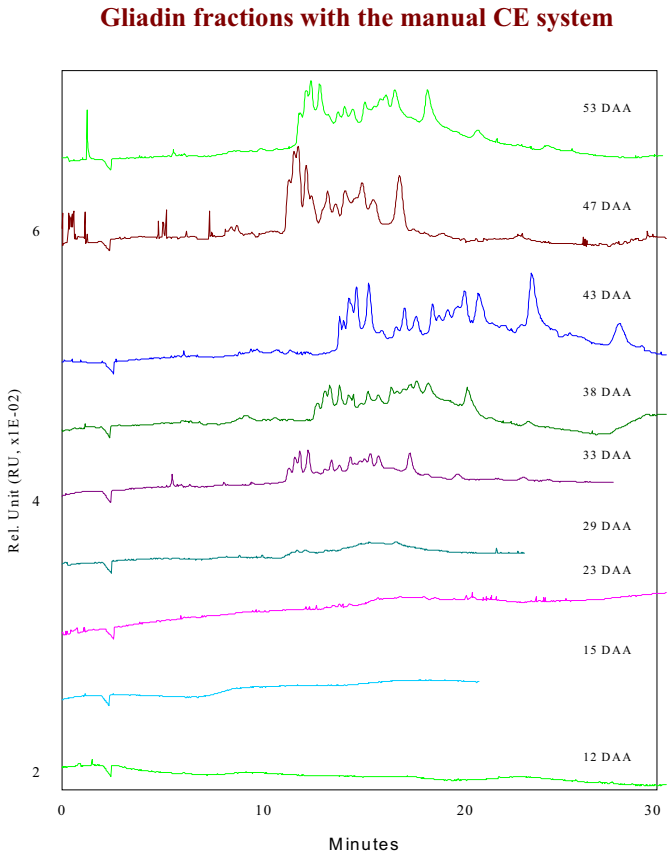

Fig. 1. Electropherograms of gliadin fractions 12–53 days after anthesis with the manual CE system. See conditions in materials and methods.

during the maturation process of wheat gliadins (*Fig. 3*), so the reason for this observation was methodological. Comparing *Fig. 1* and *3* the better reproducibility of the retention times using the automated system can be seen, too. With the automated system gliadins were detected already 23 days after anthesis. With the manual system gliadin formation was detected only 33 days after anthesis. (However, injecting four times more samples gliadins were detected 23 and 29 days after anthesis with the manual system, too (data not shown).)

Capillary gel electrophoresis was used *to study BM180*. Sample preparation and electrophoretic conditions were as described before [21]. This mammalian basement membrane protein has similar N-terminal sequence to wheat gliadins and may be an autoantigen in autoimmune diseases. Sodium-dodecyl-sulphate capillary gel electrophoresis (SDS-CGE) separates proteins according to their molecular

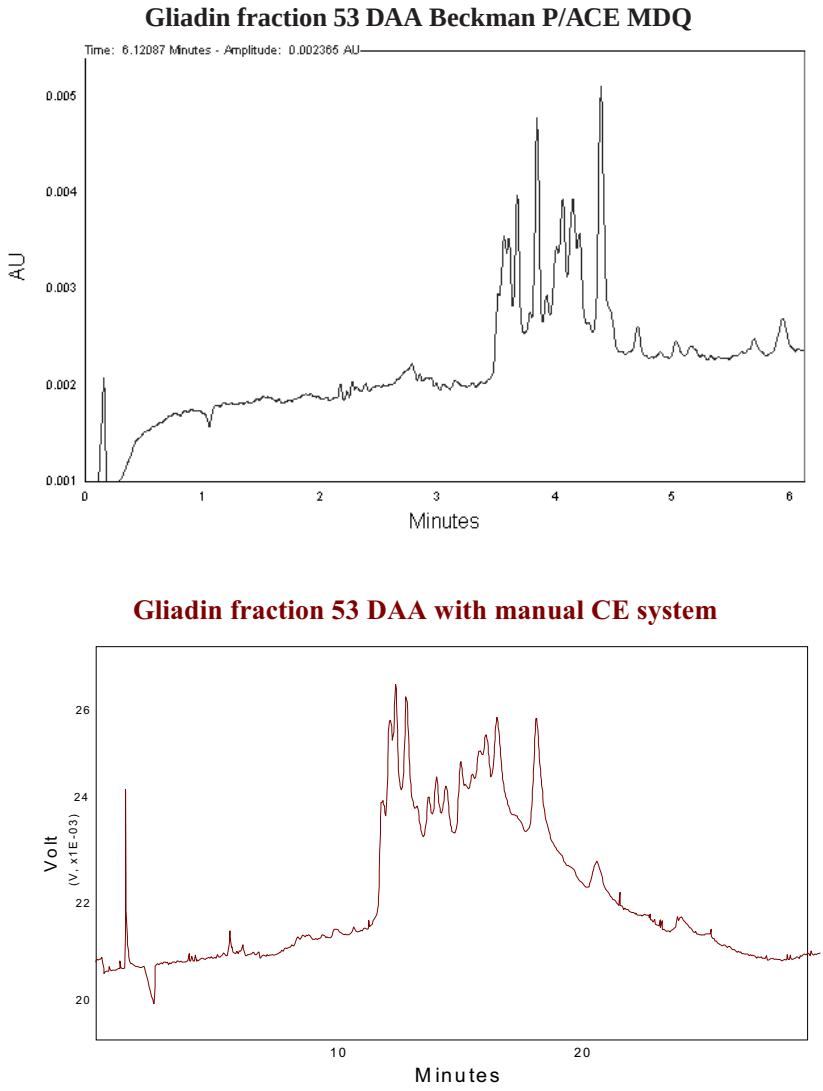

Fig. 2. Electropherograms of gliadin fractions 53 days after anthesis with the two different CE systems. See conditions in materials and methods.

mass. With this system we could follow the purification process of BM180. The separations showed that BM180 was present in only a few samples in very low concentration. The protein was not stable: it could undergo decomposition. Using reducing conditions, BM180 fell to subunits of around 60 kD. We also observed that BM180 monomers in the presence of SDS could easily form aggregates of higher

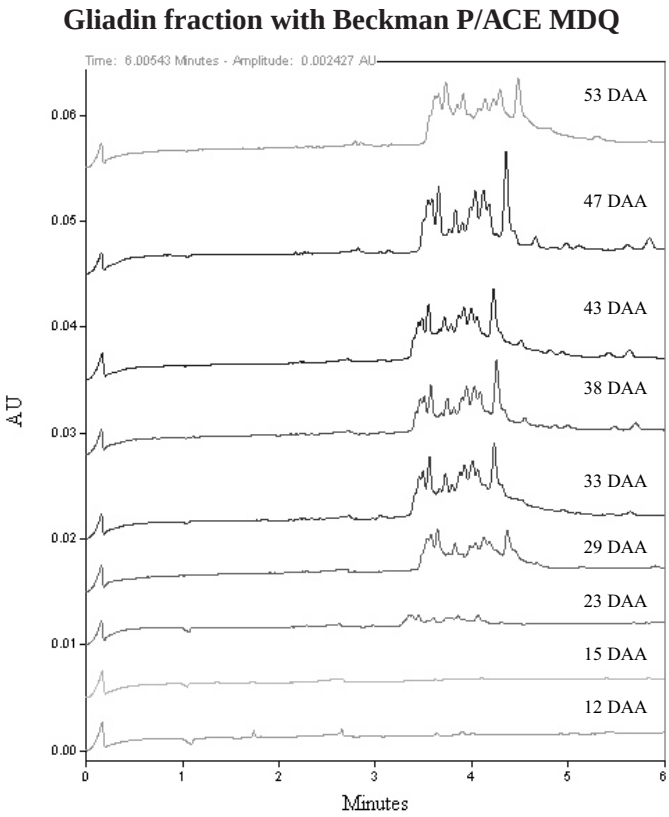

Fig. 3. Electropherograms of gliadin fractions 12–53 days after anthesis with the automated CE systems. See conditions in materials and methods.

molecular weight (120 kD or higher) [21]. (Data not shown).

4. Summary

Different modes of capillary electrophoresis were used to separate wheat and wheat analogous proteins. Capillary zone electrophoresis was used to follow gliadin formation during the maturation process. Gliadin patterns proved to be different for the various cultivars.

With the use of an automated system the separation time could be decreased and better reproducibility was achieved. The uncertain pattern changes observed with the manual system proved to be methodological.

Sodium-dodecyl-sulphate capillary gel electrophoresis was capable of the

monitoring of the purification process of a gliadin analogous protein. The size distributions of the different fractions could be determined.

Results achieved so far suggest capillary electrophoresis to be a powerful and applicable tool also for wheat and wheat analogous protein analysis. These methods can be further improved and used for wheat varietal identification and for the studying of wheat maturation process as well.

Acknowledgements

The CE experiments were supported by OTKA (National Fund of Science and Research) (T-031902 and A-143) and by a Fogarty International Research Cooperation Award (FIRCA-NIH R03). Cooperation with Dr. Gábor Pogány and Prof. Dr. Gordon W. Laurie is gratefully acknowledged. The data acquisition program of the manual system was donated by Robert Nelson (Analytical Devices Inc., CA, USA) and by Dr. Aran Paulus (Novartis, Basel, Switzerland).

References

[1] SONNTAG-STROHM, T., *J. Cereal Sci.*, **24** (1996), p. 87.
[2] ROELS, S. P. – DELCOUR, J. A.: *J. Cereal Sci.*, **24** (1996), p. 227.
[3] LARRE, C. – NICOLAS, Y. – DESSERME, C. – COURCOUX, P. – POPINEAU, Y., *J. Cereal Sci.*, **25** (1997), p. 143.
[4] BEAN, S. R. – LOOKHART, G. L., *Cereal Chem*., **74** (6) (1997), p. 758.
[5] WERNER, W. E. – WIKTOROWICZ, J. E. – KASARDA, D. D., *Cereal Chem*., **71** (5) (1994), p. 397.
[6] LOOKHART, G. L. – BEAN, S. R., *Cereal Chem*., **72** (1) (1995), p. 42.
[7] WERNER, W. E., *Cereal Chem*., **72** (3) (1995), p. 248.
[8] LOOKHART, G. L. – BEAN, S. R., *Cereal Chem*., **72** (6) (1995), p. 527.
[9] LOOKHART, G. L. – BEAN, S. R., *Cereal Chem*., **73** (1) (1996), p. 81.
[10] BEAN, S. R. – BIETZ, J. A. – LOOKHART, G. L., *J. Chrom. A*, **814** (1998), p. 25.
[11] CAPELLI, L. – FORLANI, F. – PERINI, F. – GUERRIERI, N. – CERLETTI, P. – RIGHETTI, P. G., *Electrophoresis*, **19** (1998), p. 311.
[12] BEAN, S. R. – LOOKHART, G. L., *Electrophoresis*, **19** (1998), p. 3190.
[13] ZHOU, J. – KHAN, K., *Cereal Chem*., **76** (2) (1999), p. 261.
[14] DAY, L. – GREENWELL, P. – LOCK, S. – BROWN, H., *J. Chromatogr. A*, **836** (1999), p. 147.
[15] BEAN, S. R. – LOOKHART, G. L., *J. Agric. Food Chem*., **47** (1999), p. 4246.
[16] BEAN, S. R. – LOOKHART, G. L., *J. Chromatogr. A*, **881** (2000), p. 23.
[17] BEAN, S. R. – LOOKHART, G. L., *J. Agric. Food Chem*., **48** (2000), p. 344.
[18] BEAN, S. R. – LOOKHART, G. L., *Electrophoresis*, **22** (2001), p. 1503.
[19] SCHOLZ, E. – GANZLER, K. – GERGELY, SZ. – SALGO, A., *Chromatographia*, **51** (2000), p. S130
[20] SCHOLZ, E. – GANZLER, K. – GERGELY, SZ. – SALGO, A., *Chromatographia*, **56** (2002), p. S127.
[21] SCHOLZ, E. – POGANY, G. – GANZLER, K. – LAURIE, G. W., *J. Capillary Electrophor.*, **4** (6) (1997), p. 287.
[22] WRIGLEY, C. W., *Nature*, **381** (1996), p. 738.
[23] OSBORNE, T. B., *The Proteins of the Wheat Kernel*, Carnegie Inst., Washington, 1907.