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Review

Capillary electrophoresis of recombinant proteins

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Abstract

Many naturally occurring proteins which are used therapeutically have been cloned and expressed in large quantities in bacterial, yeast or mammalian systems. Purification of these proteins by column chromatography generates high purity products with low levels of host protein contaminants. However, isoforms of the desired protein may be present at variable concentrations. Analysis of these variant forms has been enhanced by the utilisation of capillary electrophoresis (CE), a highly efficient, widely applicable technique which is increasingly used in the field of biotechnology. The role of CE in the analysis of recombinant proteins is reviewed with respect to microcharacterisation, comparison of natural and recombinant proteins, separation of mutant or variant forms and analysis of glycoforms. Examples of these applications are described and illustrated with analysis of recombinant human albumin. The rapid development of CE, further enhancing its versatility, and its use with complementary analytical techniques is also discussed. © 1997 Elsevier Science B.V.

Keywords: Reviews; Proteins; Recombinant proteins

Contents

1. Introduction

Capillary electrophoresis (CE) is a rapidly evolving separation technique with a wide range of applications including the analysis of proteins, peptides, pharmaceuticals/small molecules, oligonucleotides and DNA. CE exploits the use of narrow bore silica capillaries coupled with high voltages to enable highly efficient, highly resolving separations. The properties of electroosmotic flow and electrophoretic

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mobility are combined to separate anions, cations and neutrally charged species within the same analysis [1].

CE exhibits characteristics similar to the more conventional techniques of polyacrylamide gel electrophoresis (PAGE) and high-performance liquid chromatography (HPLC) whilst demonstrating several advantages over these methods. It is a rapid analytical technique, requiring small sample volumes and limited quantities of reagents, which is applicable to a wider range of analytes than PAGE or HPLC. However, though it was originally thought that CE would compete with HPLC as a separation technique, the two methods are now increasingly deemed complementary [2] and their performance and applications have been compared [3].

The versatility of CE is largely due to the various modes in which separations can be performed. These are capillary zone electrophoresis (CZE), capillary gel electrophoresis (CGE), micellar electrokinetic capillary chromatography (MECC), capillary isoelectric focusing (CIEF) and isotachophoresis. The scope of CE has increased further due to recent advances in several areas, i.e., a wide range of capillary coatings and size separation media [4,5]; an extensive selection of modifiers [6] and chiral selectors [5]; improved design of capillary surfaces (such as etching [7]) and enhanced instrumentation/detection methods [8].

In the manufacture of recombinant proteins, product quality may be reduced by the presence of closely related protein variants resulting from post-translational modifications. These are frequently of one amino acid only and their analysis by HPLC–PAGE is often difficult. The high resolution and efficiency of CE is suited to recombinant protein analysis, as is ease of automation which facilitates the development of validatable routine analyses for use in quality control. These features increase throughput of samples and minimise operator intervention. Therefore, CE is used for process monitoring, purity assessment and product quality evaluation as well as for characterisation of recombinant proteins.

Many reviews have been written encompassing different areas of CE development such as fundamental theory and instrumentation [9], modes of CE [10,11], detection methods [12,13], clinical analysis [14] and pharmaceutical applications [6]. This review will discuss the increasing utilisation of CE in biotechnology, highlighting its use in the analysis of recombinant proteins and concentrating principally on CZE on uncoated and coated silica capillaries. Some examples of CGE and micropreparative applications are also included. CGE and CIEF have been reviewed in more detail elsewhere [15] and are only briefly discussed here.

2. CE in microcharacterisation

The high resolution of CE allows rapid separation of closely related molecules which may be difficult or impossible by HPLC methods. Therefore, CE has been used in the routine analysis of many different recombinant proteins and peptides, improving on current HPLC methodology. CE methods have been developed for the analysis of peptide maps [16–19], the determination of microheterogeneity within protein species [20], the analysis of protein glycoforms [21] and the determination of the carbohydrate microheterogeneity of glycosylated proteins [22].

2.1. Peptide map analysis

CE is applicable to the analysis of peptides including those generated by enzymatic or chemical cleavage, i.e., peptide maps. Peptide map analysis is the only method for determining changes in oxidation, deamidation and the primary sequence of proteins by HPLC, but such differences may be determined directly using CE e.g., the identification of amino (N)-terminally degraded species (see Section 2.2, Fig. 2). Even so, the most comprehensive information comes from the analysis of peptide maps and is an important requirement for recombinant proteins from a drug regulatory standpoint to demonstrate structural equivalence with their natural counterparts. CE methods are being developed for the analysis of peptide maps, and those to date demonstrate that the advantage in using CE over HPLC is a greater throughput of samples due to a drastically reduced analysis time from hours to minutes. The analysis of recombinant human growth hormone (rhGH) tryptic maps by CE is a well studied example [19]. The separation of the nineteen peptides gener-

ated from a non-reducing trypsin digest of rhGH by HPLC and CE demonstrates that CE provides better resolution than HPLC, implying it is a useful tool in the routine analysis of peptide maps. The order in which the peptides eluted by CE was different from HPLC due to the difference in the separation method. This variation has also been noted by other workers comparing the two methods [16]. This can be advantageous, either by further resolving peptides or confirming the homogeneity of single peaks from HPLC. Thus, the complementary use of CE and HPLC provides a more complete peptide map analysis.

CE can also be used for the structural and physical determination of proteins/peptides by direct analysis of migration times [23]. In this work, the migration time of a peptide allows theoretical determination of its physicochemical properties. Conversely, the use of such theoretical methods may allow prediction of the migration time of a peptide by relating its properties to the separation conditions. A system for predicting peptide migration in CE separations has been developed [24] which uses the pK_a of a peptide at a specific pH to calculate its mobility at a given voltage. It has been shown that this method is reliable for predicting the electropherogram of peptides of up to 14 amino acids in length. The further development of these methods will allow enhanced analysis of more complex peptide maps. In the case of reduced recombinant human albumin (rHA), a trypsin digest generates more than 80 peptides, most of which can be resolved by HPLC although the HPLC analysis time is in excess of two hours. Though the complete separation of all these peptides using CZE has not yet been achieved in our laboratory, a non-optimised separation of a non-reduced rHA tryptic digest (in which fewer tryptic peptides were generated as the disulphide linkages were not cleaved prior to digestion) demonstrated resolution of the majority of the 65 peptides expected (Fig. 1). The use of a system for predicting peptide mobilities from their ionisation constants could aid in the rapid development of a separation method for such peptide maps and could be applied to other types of peptide analysis.

2.2. Protein analysis

Analysis of a digested protein is suitable when close scrutiny of the primary structure is required. However, such methods take considerable effort to develop and may only be used to support analysis by other techniques. The highly resolving nature of CE can be usefully applied to the analysis of native

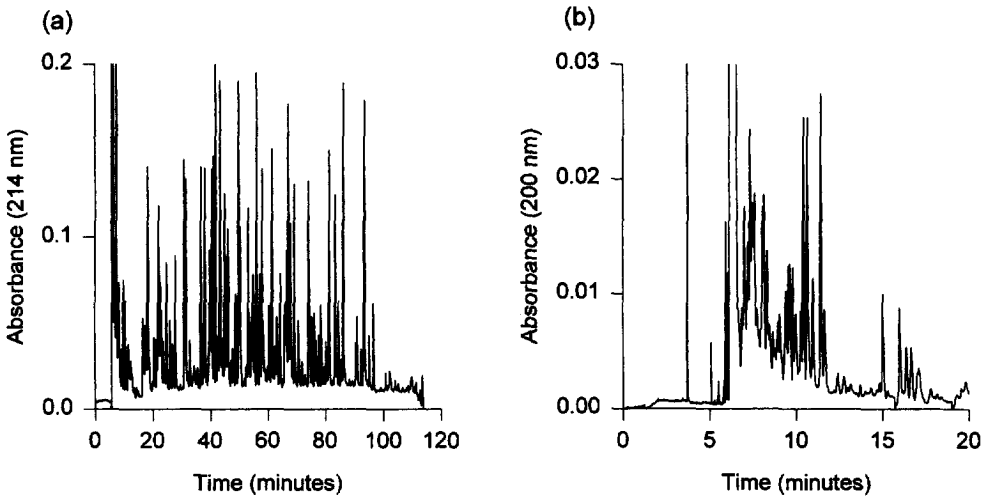

Fig. 1. Comparison of rHA tryptic digest separations by HPLC and CZE. rHA was digested with trypsin (a) reduced and alkylated and peptides generated separated by RP–HPLC using a Vydac C_{18} column with an aqueous acetonitrile–trifluoroacetic acid gradient and (b) non-reduced and peptides separated by CZE using an uncoated capillary with 100 m*M* phosphate buffer pH 2.5 at 20 kV.

proteins. There are many examples where protein modifications have been analysed using CE, e.g., acetylation of insulin [25], deamidation/succinylation of rhGH [26] and oxidation/reduction of human serum albumin (HSA) [20].

It is possible to achieve good separations of proteins on bare silica uncoated capillaries. However, in many cases, it is necessary to minimize adsorption of the protein to the capillary surface by either inclusion of buffer additives, or capillary coating to render silanol groups inert, thereby altering sample–capillary wall interactions. Many compounds have been used to enhance CE separations, some of which are listed in Table 1. Several reviews also discuss the use of buffer additives comprehensively [38–41].

Coated capillaries impart a reduction of both electroosmotic flow (EOF) and the possibility of protein adsorbing to the capillary surface, thereby improving separation efficiency. The use of such coatings has been reviewed previously [42]. Another advantage of coated capillaries is that proteins may be separated close to their isoelectric points (p*I*) at which subspecies within a single population can be resolved. At pH 5.2 HSA can be separated into eight subspecies using a neutral coated capillary, whereas only one peak is observed using an uncoated capillary [20]. Thus, the enhanced resolution of HSA separation using a coated capillary compared to that on an uncoated capillary is demonstrated (Fig. 2).

Not all proteins adsorb to uncoated capillaries in this way, as this is related to the size and the nature of the protein being analysed. CZE has been shown to be an invaluable tool in the production of recombinant human insulin where its use on uncoated capillaries, in conjunction with HPLC systems, has allowed subtle changes in the molecular structure of insulin to be analysed during manufacture [43]. The workers also used the technique to identify contaminating species within each stage of the insulin production process. CZE on uncoated capillaries has been used to monitor deamidation of insulin [44] and the methodology has been compared to other analytical techniques, i.e., ion-exchange chromatography and reversed-phase (RP)-HPLC. CZE compared favourably with these methods, demonstrating better resolution of the deamidated product.

Size determination of proteins by CGE is an area that has developed rapidly in recent years. The evolution from the use of cross-linked acrylamide gel systems [45] through linear polyacrylamide [46] to a variety of alternative sieving matrices, e.g., pullulan [47], dextran [48], poly(vinyl alcohol) [49] and agarose [50], has broadened the scope of molecular mass determination in capillaries. This development

Table 1
Buffer additives for optimisation of peptide and protein separations

Effect of buffer additive	Type of additives used	Example(s) of additive used	Possible applications	References
Increased selectivity	Ionic and non-ionic surfactants, cyclodextrins, metal ions, high ionic strength buffers, zwitterions	Sodium dodecyl sulfate, cetylamminonium bromide, Tween 20, dimethyl-β-cyclodextrin, Cu^{2+}, 0.5 *M* sodium phosphate, betaine, sarcosine	Peptide separations, protein analysis, glycoprotein analysis	[27–32]
Reduced analyte–wall interactions	Non-ionic surfactants, divalent amines, high ionic strength buffers	Polyoxyethylene-10, 1,4-diaminobutane, 1,5-diaminopropane, 0.25 *M* sodium phosphate	Peptide separations, protein analysis, glycoprotein analysis	[27,31,33,34]
Regulation of EOF	Organic solvents, non-ionic surfactants	Methanol, acetonitrile, cetylammonium bromide	Peptides	[28,35,36]
Dynamic wall coatings	High ionic strength buffers, zwitterions, divalent amines, polymers	0.5 *M* sodium phosphate, 0.5 *M* potassium phosphate, sarcosine, polybrene	Proteins	[31–33,37]

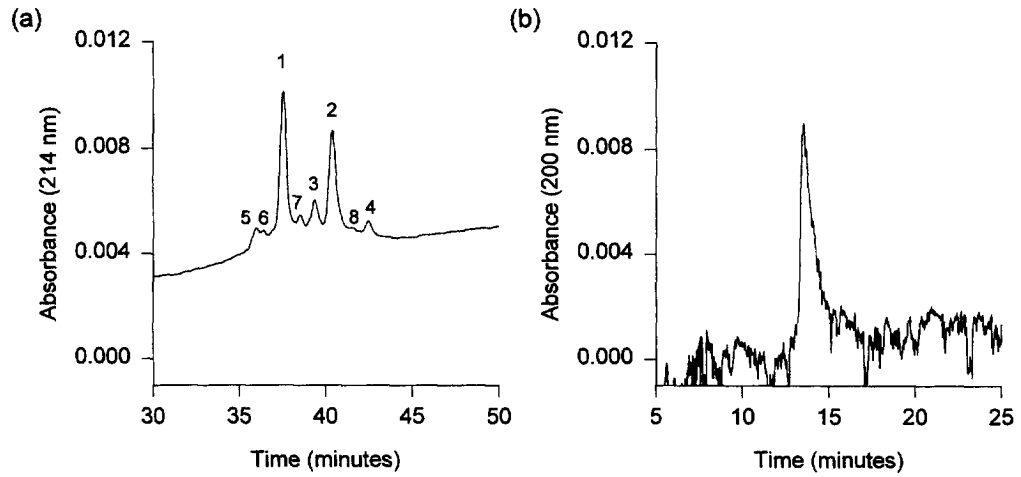

Fig. 2. Comparison of HSA separated on a neutral coated and an uncoated capillary. HSA was separated at pH 5.2 on (a) a neutral coated capillary resolving eight peaks which have been identified as follows: (1) intact monomer with free thiol; (2) intact monomer with blocked thiol; (3) *N*-terminally degraded monomer with free thiol and (4) *N*-terminally degraded monomer with blocked thiol; peaks 5 to 8 unidentified, and (b) an uncoated capillary which exhibits poor resolution. Separation conditions for (a) 20 m*M* citrate MES buffer at 18.5 kV and (b) 50 m*M* phosphate buffer at 20 kV.

has partly overcome problems originally observed with CGE, e.g., bubble formation and lack of reproducibility, and the continued improvement in CE instrument design should improve further the robustness of CGE for use as a routine analytical technique.

In our laboratories, CGE using a commercially available kit (based on a non-acrylamide, SDS-containing replaceable gel matrix) has been investigated for the quantitation of rHA in *Saccharomyces cerevisiae* culture supernatant (Fig. 3). The rHA monomer peak is well resolved from other culture supernatant components, e.g., yeast proteins, the majority of which are smaller and so migrate faster than the albumin. Though partially successful, development of the technique into a routine analytical assay has been hindered by problems associated with instrument robustness (precipitation of buffer components, reduction of optics efficiency due to viscosity of the gel matrix) and irreproducibility of rHA quantitation. These problems may be overcome by both improved instrument design and the use of alternative polymer solutions.

A further consideration in method development when using coated capillaries (either for CGE or CZE) is accounting for potential losses of analyte due to adsorption onto the capillary wall. Mass

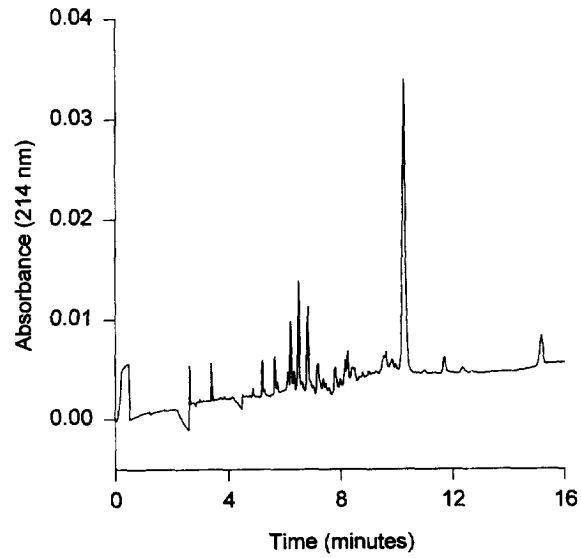

Fig. 3. Analysis of rHA-containing *Saccharomyces cerevisiae* culture supernatant by CGE. A size-based separation of *Saccharomyces cerevisiae* culture supernatant was performed using an SDS containing replaceable gel matrix (eCAP SDS 14-200 gel kit, Beckman Instruments, UK). The resolution of rHA monomer (10.2 min) from other centrate components is demonstrated.

balance calculations of the quantity of sample eluted from the capillary compared to the quantity of sample loaded can be performed to indicate if protein adherence is occurring despite the capillary coating (which may be incomplete or unstable). This aspect could contribute to peak area variability and, therefore, affect reproducibility and should be investigated if a quantitative separation is being performed.

CE is proving particularly successful in determining the heterogeneity of carbohydrate moieties attached to glycoproteins, i.e., protein glycoforms. MECC has been employed in this area [51] as has CIEF [15,52]. CZE with buffer additives/modifiers has also been widely applied to glycoprotein analysis and is discussed in greater detail in Section 5 of this review.

Protein folding and thermal denaturation have been studied by CE [53–55]. Percentages of intermediate structures in the thermally induced unfolding of lysozyme at low pH have been reported [55]. The changes in the structure of lysozyme resulted in both peak broadening and other peaks being identified, which related to intermediate species. Thermodynamic parameters for the unfolding process could be calculated from relative levels of these species which compared favourably with those calculated from calorimetric studies. Similarly the intermediate states of trypsinogen folding have been analysed using CE [53]. In this work, the data generated were compared to data from size exclusion chromatography and biological activity assays, and showed good correlation. CE enabled the rapid identification of possible unstable populations of molecules, replacing lengthy calorimetry studies. Also, the transition states in the conformational changes can be identified, contributing to a broader structural knowledge of the proteins involved.

2.3. Micropreparative CE

As with HPLC separations, peak matching in CE is insufficient for the identification of a protein of interest, necessitating fraction collection, i.e., micropreparative CE [56]. The collected material can then be subjected to mass analysis or *N*-terminal sequence analysis [57]. Collection of fractions from CE is more challenging than from HPLC as the volumes involved are extremely low (approximately 100 nl or less). Collection of material is also hindered by diffusion of separated analytes when the current is disconnected to change to the outlet collection vial. Despite this, on commercial CE instrumentation it is possible to collect fractions into 10 μl of separation buffer, though this dilutes the analyte greatly which may result in detection problems with post-separation detection methods. It has been reported that utilising D_2O in the separation buffer reduces the amount of diffusion and aids in peak collection [58].

However, this aspect of CE may be relatively short-lived due to the rapid development of on-line analytical techniques, e.g., CE–mass spectrometry (CE–MS) which eliminate the need for fraction collection. The use of CE as a separation system for mass spectrometry (MS) enables peaks to be identified directly from the CE column. This also permits CE–MS–MS to be performed for peptide sequence determination. In future, the collection of fractions for *N*-terminal sequencing may be directly onto a polyvinylidene difluoride membrane in a system similar to the Perkin-Elmer ABI 173 Microblotter, which utilises capillary liquid chromatography and a nanolitre fraction collection method.

3. Natural versus recombinant protein comparisons

CE can be utilised along with other characterisation techniques to compare the structure of recombinant proteins to their natural counterparts. Recently Knüver-Hopf and Mohr [59] analysed natural (nIL-2) and recombinant interleukin-2 (rIL-2) using both SDS–PAGE and CZE. Using a phosphate buffer system at pH 2.5 nIL-2 was separated into three naturally occurring forms (non-glycosylated, glycosylated-monosialyated, glycosylated-disialylated) by CZE, which were also identified by reducing SDS–PAGE. However, relative peak heights of the three components differentiated by CZE varied between two alternative preparation techniques for nIL-2. The direct UV quantitation of CZE allowed calculation of relative proportions of the three forms, which was not possible from the degrees of band intensity by SDS–PAGE. rIL-2 exhibited two peaks by CZE analysis using the same conditions as above, but only

one band by reducing SDS–PAGE. The authors suggested that these peaks may indicate slight conformational differences in the usual non-glycosylated recombinant IL-2 form. This demonstrates the high selectivity of CZE for the separation of proteins with a single charge difference only.

CZE has been used in our laboratory to compare HSA derived by blood fractionation with rHA produced in yeast [60]. Due to problems of adsorption of albumin to the walls of uncoated silica capillaries, a neutral coated capillary was used, enabling separation to be performed at a pH close to the p*I* of albumin i.e., pH 5.2. This achieved optimum resolution of HSA subspecies, four out of eight of which have been identified [20]. The two main peaks were identified as intact monomer with a free thiol group at cysteine 34 and monomer with the thiol group blocked with cysteine. Also identified were the *N*-terminally degraded forms of these species (Fig. 2). CZE demonstrated better resolution of these four species than was achieved by electrospray ionisation mass spectrometry (ESI–MS) as previously reported [61]. In contrast to the heterogeneous CZE profile obtained for HSA, rHA demonstrated a more homogeneous composition, constituting one main peak corresponding to intact monomer and low levels of some of the as yet unidentified peaks seen in HSA (Fig. 4).

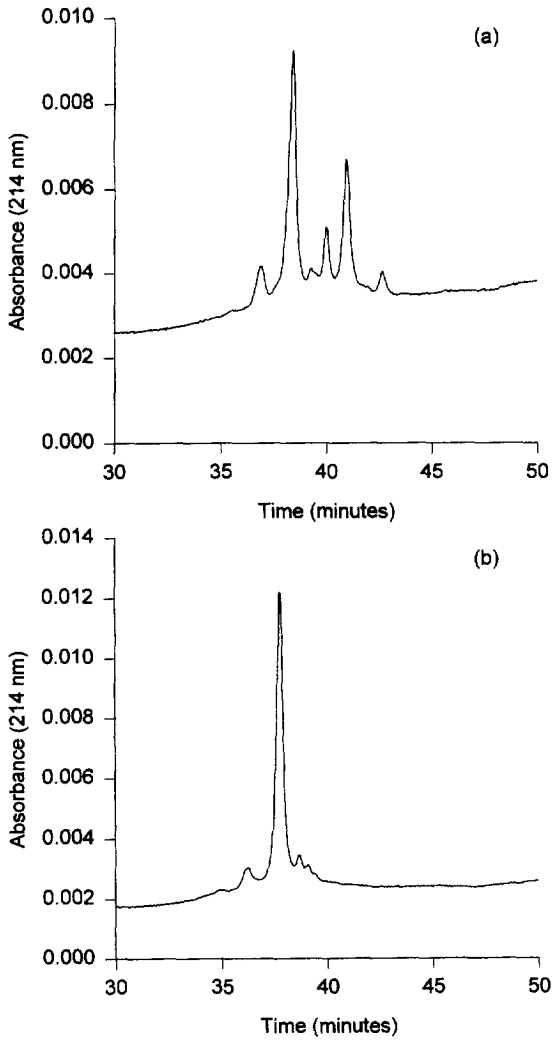

Fig. 4. Comparison of HSA and rHA separations on a neutral coated capillary. HSA (a) and rHA (b) were separated on a neutral coated capillary at pH 5.2, close to the p*I* of albumin, to achieve optimum resolution of subspecies. Peak identities and separation conditions are as specified in Fig. 2a.

4. Separation of mutants/variants

CE has been used successfully to distinguish between mutants or variants of a number of recombinant proteins. Kálmán et al. [62] employed CZE to separate twelve Staphylococcal nuclease variants produced by site directed mutation at one amino acid residue only. A capillary coated with an inert, hydrophilic layer was used in conjunction with suitably selected oligoamino buffers between pH 2.8 and pH 9.5. This free solution system resolved variants which exhibited small but well defined structural differences.

Zhao et al. [63] used CZE to study ferritin protein variants with both uncoated capillaries at pH 8.2 and coated capillaries at pH 2.0. The former conditions allowed investigation of the fully assembled ferritin properties whereas the latter examined the characteristics of individual ferritin subunits. The authors state that the CE methodology enabled the further elucidation of ferritin behaviour using a faster, more reliable and user-friendly system than had previously been employed to investigate ferritin subunit association, i.e., that of anaerobic gel electrophoresis.

Analysis of recombinant insulin-like growth factor I (rIGF-I) variants were analysed by Nashabeh et al. [64] and utilised selectivity by hydrophobic inter-

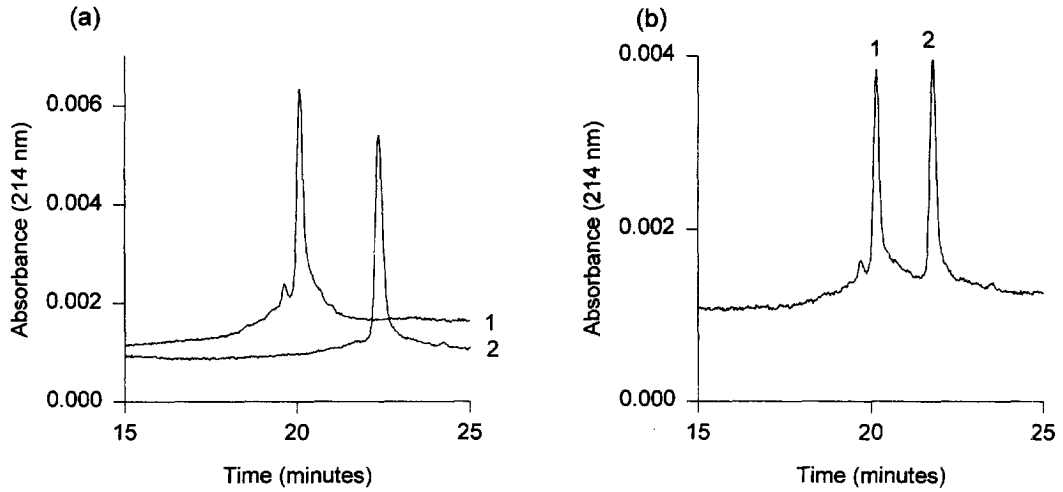

Fig. 5. Separation of rHA monomer from *N*-terminally truncated (NTT) rHA on a neutral coated capillary. Intact rHA monomer (1) was separated from NTT rHA (2) either in separate analyses (a) or within the same analysis (b) using a neutral coated capillary with 20 m*M* citrate MES buffer pH 5.5 at 18.5 kV, demonstrating baseline resolution of the two components.

action in CZE. A neutrally coated capillary was used to minimise both EOF and analyte–wall adsorption. This was coupled with mixed aqueous-organic buffers containing variable amounts of zwitterionic detergent acting as a hydrophobic selector. This system achieved resolution of four rIGF-I variants, with closely related mass-to-charge ratios, within 16 minutes. The molecular masses of these and a fifth variant (not resolved by CZE alone) were correctly assigned by on-line CE mass spectrometry (CE–MS). This study illustrates the compatibility of CZE with on-line CE–MS and the advantage of the method for rapid analysis of process samples.

In our laboratory we have performed CZE using a neutral coated capillary to separate intact rHA from a biosynthetically made *N*-terminally truncated (NTT) rHA, from which the amino acid Asp had been omitted from the *N*-terminus. This separation was performed at pH 5.5 which demonstrated excellent baseline resolution of the two components (Fig. 5).

5. Glycoform analysis

There are many examples of the use of CE in the separation of recombinant protein glycoforms. Rudd et al. [65] separated glycoforms of bovine pancreatic ribonuclease B (RNase B) with different numbers of mannose moieties by CZE on a fused-silica capillary with sodium phosphate–sodium tetraborate buffer at pH 7.2 containing 50 m*M* SDS. CZE analysis of RNase B exoglycosidase digestions was used to generate a glycoform ladder, which was subsequently employed in the determination of natural RNase B glycoform heterogeneity. This allowed individual glycoforms to be identified and studied further, which demonstrated that glycosylation affected the overall dynamic stability of the molecule and also modified the functional activity of the enzyme.

Watson and Yao [66] also used CZE to resolve the six major glycoforms of recombinant erythropoeitin. Their separation system included the addition of 1,4-diaminobutane to reduce EOF and urea to achieve optimum resolution and improve peak shape. It was demonstrated that separation was due to differences in electrophoretic mobility of the various glycoforms according to the number of sialic acid moieties present. Therefore, CZE showed equivalent separation to gel isoelectric focusing with the advantage of being quantitative.

The same authors again employed diaminobutane as a buffer additive for the separation of recombinant granulocyte-colony-stimulating factor glycoforms [67] which were also resolved on the basis of number of sialic acids present. As previously, the comparison between CZE and gel IEF was made,

illustrating the advantages of the former for glycoform analysis, i.e., its speed, simplicity and quantitative nature. Diaminobutane was also successfully used by Klausen and Kornfelt [68] in the separation of recombinant coagulation factor VIIa glycoforms, indicating that this particular additive is extremely useful for the analysis of sialic acid containing glycoforms.

Detergents other than SDS have been utilised as buffer additives for glycoform separations. Yim [69] used Triton X-100, together with ϵ-aminocaproic acid as a solubilising agent, in the CZE separation of recombinant tissue plasminogen activator glycoforms. This separation was of a complex mixture of approximately fifteen glycoforms and baseline resolution was not achieved by the CZE methodology, though this was performed on a short length capillary (20 cm) and may be improved by using a longer length capillary. In this study CZE was compared directly to CIEF, the latter providing a better resolved separation.

Buffer additives are useful but not essential to all glycoform separations. Yim et al. [70] analysed nine glycoforms of recombinant human bone morphogenetic protein 2 (rhBMP-2) by CZE using a simple phosphate buffer system at pH 2.5 with no additives. However, the authors demonstrated rhBMP-2 has fifteen glycoforms and only those with differing numbers of mannose residues could be separated. Glycoforms with the same number of mannose residues (i.e., stereoisomers) could not be separated. As the total number of glycoforms present for some of the earlier examples has not been specified, it is not possible to judge the success of this separation with respect to those containing buffer additives. However, these results suggest that CZE at low pH may be a suitable method for the analysis of complex protein glycoform mixtures.

6. Future advances in CE

Recently capillary electrochromatography (CEC) [71] has come to the forefront of CE method development. Although still in its infancy, this technique already allows similar separations to HPLC with equivalent column loading and detection. CEC uses EOF to induce a flow-rate through capillaries packed with either unmodified silica or chemically derivatised matrices. The main advantage of CEC is that the flow-rate, which is governed by the rate of EOF, allows small bead sizes, i.e., 3 μm, to be used in the packing material. This, together with the use of short columns, may markedly reduce analysis times. Columns, which have only recently become commercially available, can be packed with a wide range of materials e.g., 3 μm Hypersil C_{18}, 3 μm Spherisorb ODS1. These allow rapid separation of compounds, with the number of theoretical plates in the region of 10–100 times greater than that of HPLC columns. As with many new techniques, there is a reluctance to use CEC as researchers are uncertain as to its advantages over HPLC methods. However, as knowledge of the area increases and CEC columns become more widely available, hesitancy regarding the utilisation of the technique may be overcome.

To aid in the analysis of proteins, development of couplings for detector systems which include ESI–MS [72] and fast atom bombardment mass spectroscopy (FAB–MS) [73], has enhanced the quality of the data which can be generated from CE. There is much interest in the use of CE–MS for the routine analysis of pharmaceuticals and peptides. CE is particularly well suited for ESI-MS due to the low flow-rate caused by EOF (nl/min). Such low flow-rates enable the analyte of interest to be maintained in the electrospray source for a considerably longer time frame than is the case for narrow bore HPLC methods. This allows the collection of higher quality data and ultimately more time to perform peptide sequencing by ESI-MS. CZE–ESI-MS has been used for the analysis of recombinant bovine and porcine somatotropins (rbSt and rpSt) [74] demonstrating average molecular masses nearly identical to the theoretical values for these two proteins. In addition, CZE separated mono and dideamidated and monoacetylated species and ESI-MS detected an analogue and a truncated homologue of rpSt, as well as mono and dioxidised homologues in both rbSt and rpSt. This study emphasised the enhanced analytical capacity of coupling the CZE and ESI-MS methods, as the individual techniques could not detect all impurities. The usefulness of CZE–ESI-MS as a tool for rapid characterisation of recombinant proteins is stressed.

7. Conclusions

This review has illustrated the versatility and applicability of CE, especially with respect to recombinant protein analysis. That it is a powerful tool, either on its own, or coupled with other techniques, for characterisation and elucidation of structural information is demonstrated. The rapid development of the field, some areas of which have been discussed, will further increase the potential use of CE in biotechnology as well as in other areas of the biological sciences.

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