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Comparison of standard capillary and chip separations of sodium dodecylsulfate–protein complexes

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Abstract

Conditions for converting a set of five standard proteins to electrochemically active sodium dodecylsulfate (SDS) complexes were worked out with the aim of using such complexes for conductivity detection with a a chip electrophoresis system. The results obtained were compared with standard capillary electrophoresis (37 cm (effective length 30 cm)×75 μm I.D. capillary, 10 kV, negative polarity at the inlet). The chip separations were run at 500 V per chip (100 V/cm) as compared to the standard capillary arrangement, which was run at 266.6 V/cm. For the capillary set-up the protein complexes were prepared in aqueous solution (Milli-Q water) made 10 m*M* with respect to SDS. If the SDS concentration was increased to 50 m*M*, the separation in the capillary was incomplete. On the other hand with the chip system both approaches yielded acceptable results. The chip separations were slightly (but not distinctly) shorter and offered better separations than the standard set-up. The concentration of the surfactant used for the preparation the complexes results in alternations of the elution sequence, which is preserved if the chip separation is used instead of the capillary set-up. Apparently the full capacity of protein–SDS binding is not exploited for the preparation of the adducts.

Keywords: Complexation; Chip technology; Proteins; Sodium dodecylsulfate

1. Introduction

Microfluidic systems in which separation is effected by electrokinetically driven flow have progressed considerably since 1997 with the perspective of developing a laboratory-on-a chip system (for a review see Ref. [1]). The key issues are fabrication of the chips, microchip layout and detection. Regarding the materials used, glass [2,3], poly(dimethylsiloxane) [4] and polymethacrylate [5,6] are the most frequently used. None of these materials is ideal and all of them impose considerable limits upon detection and channel geometry, not to mention, for example, sealing problems during chip channel preparation. Though the most frequently used chip manufacturing technology is photolithography, the easiest method of manufacture is the molding process for polymer systems as reported in Ref. [4].

As regards detection, laser induced fluorescence appears the most common method, however, it is applied mainly to the analysis of DNA and DNA fragments [7,8] where single molecules [9] can be detected. The other frequently used method is the use of electrochemical and conductivity detectors as demonstrated in Refs. [10,11]. It is, perhaps, not

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surprising that MS is another suitable candidate, particularly in protein and peptide separations [12,13]. All in all the need for an ultra sensitive non-specific method of detection is quite obvious.

In addition to the problems outlined above, current progress in applications of chip electrophoresis suffers from the fact that to the best of our knowledge, there is no universal chip electrophoresis system commercially available. A chip electrophoresis system is now marketed by Agilent, however it is designed for routine applications only. A single purpose system has been introduced by Shimadzu aimed at DNA fragment analysis. Its application to other analytes (particularly proteins) bears a hidden danger of clogging the chip, the replacement of which is exceedingly costly.

As far as protein analysis is concerned, chip electrodriven separations of peptides/proteins is currently limited to model mixtures [14–20]. Except for Refs. [18,19] in which UV–laser-induced fluorescence detection was used, all the others exploited MS for detection. Guijt et al. [22] recently reported four electrode capacitively coupled conductivity detection for short chain peptides. They emphasised that conductivity detection is a relatively universal detection technique the main advantage of which is that it is nearly independent of the detection pathlength and, consequently, is suitable for chip-based analysis (peptides separated were Lys–Pro–His–Gly–Glu–Ala–Asp–Asp–Ser–Lys and Glu–Lys–Phe–Glu–Lys–Ser–Ala–Asn–Val–Asp–Gly, and exhibited isoelectric points of 5.38 and 4.87, respectively). The peptides were separated within 60 s and the peak of the endoosmotic flow appeared at 1400 s. Regarding separation conditions, the background electrolyte was 50 m*M* phosphate, pH 2.5, made 2 m*M* with respect to SDS, which was claimed to reduce bubble formation during the run. The field strength was 300 V applied over the 6-cm long channel (rectangular channel 20×70 μm manufactured on a glass wafer) with 3-s injection time. The achieved plate number was 3500 with the detection limit ~0.2 m*M* (for a conventional CE–UV system the detection limit lies in the low μ*M* range). The authors claim the low sensitivity of the system is due to the inadequacy of the insulating layer and poor (highly noisy) electronic set-up.

2. Materials and methods

A laboratory-made chip electrophoresis set-up described in our previous communication was used in the experiments [21]. Comparative results (standard electrophoretic separation) were obtained with a Beckman PACE 5000 instrument which was equipped with a 37.5 cm (effective length 30 cm)× 75 μm untreated fused-silica capillary. The chips used were produced experimentally by Krejci Engineering (Tisnov, Czech Republic) and were made of fused (1600–1800 °C) corundum ceramics (99–99.9% Al_2O_3 declared purity, purchased from Kyocera, Kyoto, Japan) equipped with silver made connections and gold contacts installed by means of a corund-phenolic resin (for details see Ref. [21]). The separation channel was rectangular, inner dimensions 20×50 μm, and 5 cm long. No thermostatting was used in the chip experiments; comparative results with standard capillary electrophoresis were obtained at 20 °C. Samples were introduced by overpressure (7.5 MPa for the chip version and 3.5 MPa for the conventional separation mode).

At the beginning the capillary systems were washed with 0.1 *M* NaOH followed by a water and run buffer rinse (2 min each). Phosphate buffers of the concentration and pH indicated in the Results were used as background electrolytes either directly or containing the specified concentration of sodium dodecylsulfate (SDS).

Detection in the standard equipment was done by UV at 214 nm; chip electrophoresis worked with conductivity detection (conductometer Gryf 156, Gryf, Havlickuv Brod, Czech Republic) equipped with capacitance shielding from the high voltage applied. Standard electrophoretic separation was at 10 kV (266.6 V/cm) while in the chip electrophoresis system the voltage applied was 0.5 kV (100 V/cm). Working at a higher voltage in the chip electrophoresis system was precluded by bubble formation even in the presence of SDS, which is claimed to abolish this effect [22].

All chemicals used (proteins, peptides and buffer components) were of p.a. or highest purity available. Standard proteins/peptides: cytochrome *c* (rel. mol. mass 12 500), chymotrypsinogen A (rel. mol. mass 25 000), bovine serum albumin (rel. mol. mass

68 000), catalase (rel. mol. mass 240 000) were purchased from Boehringer (Mannheim, Germany); standard protein trasferrin (rel. mol. mass 76 000–81 000) was from Sigma (St. Louis, MO, USA). Buffer components were obtained from Lachema (Brno, Czech Republic). SDS was obtained from Merck (Darmstadt, Germany). All solutions were prepared in Milli-Q water (Millipore, Bedford, MA, USA) and before analysis were filtered using a 0.45-μm Millex-HV filter (Millipore).

For runs with SDS the respective amount of SDS was added to the Milli-Q water dissolved sample to achieve a final concentration of 10 or 50 m*M*.

3. Results and discussion

Separations achieved by the chip electrophoresis system were compared to the results obtained by standard capillary electrophoresis. However, because of the detection limitations of the chip electrophoresis system (conductivity detection vs. UV detection in the standard set-up) the electromigration behavior of the SDS–protein complexes was studied in the preliminary stage in order to work with optimised conditions in the chip-based separations.

Separation in a bare silica capillary at 10 kV in pH 2.5 phosphate buffer (50 m*M*) in the absence of the surfactant in the background electrolyte is presented in Fig. 1. Individual model proteins were brought in front of the detector window (UV, 214 nm) in the following sequence: cytochrome *c*, albumin, catalase, transferrin and chymotrypsinogen A. The selectivity of the system was fair and the last four model proteins (albumin, catalase, transferrin and chymotrypsinogen A) were nearly separated to the baseline.

In order to be able to detect the analytes by conductivity in the chip operational mode, the proteins investigated were converted into SDS complexes (in the standard capillary mode runs the detection was, however, done by measuring UV absorbance at 214 nm as specified in Materials and methods). After being complexed with the anionic tenside, the analytes became highly negatively charged and, consequently, the separation had to be done in the negative polarity mode (cathode at the injection side of the capillary).

Separation in a bare silica capillary in pH 2.5 phosphate buffer made 2 m*M* with respect to SDS (separation at 10 kV, 20 °C) resulted in a profile shown in Fig. 2. The sample compounds were dissolved in Milli-Q water made 10 m*M* with respect to SDS. The elution order of the model proteins was changed and individual members were brought in front of the detector window in the following sequence: chymotrypsinogen A, cytochrome *c*, catal-

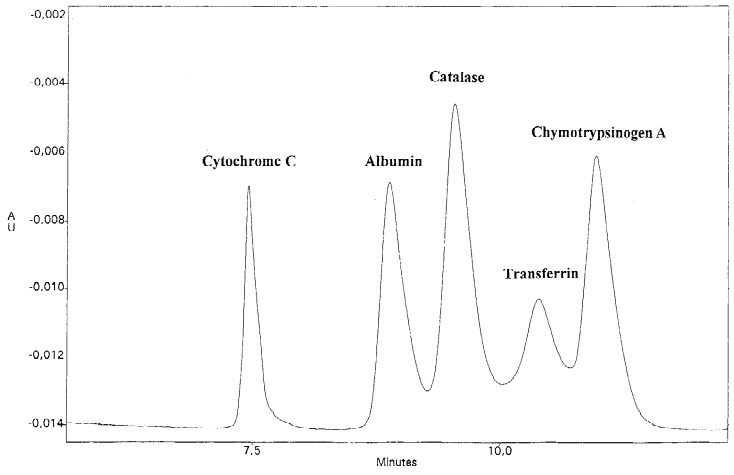

Fig. 1. Standard capillary electrophoresis of model proteins in phosphate buffer (50 m*M* NaH_2PO_4, pH 2.5) at 10 kV, 25 °C. No SDS present in the background electrolyte. Positive polarity at the inlet. UV detection at 214 nm.

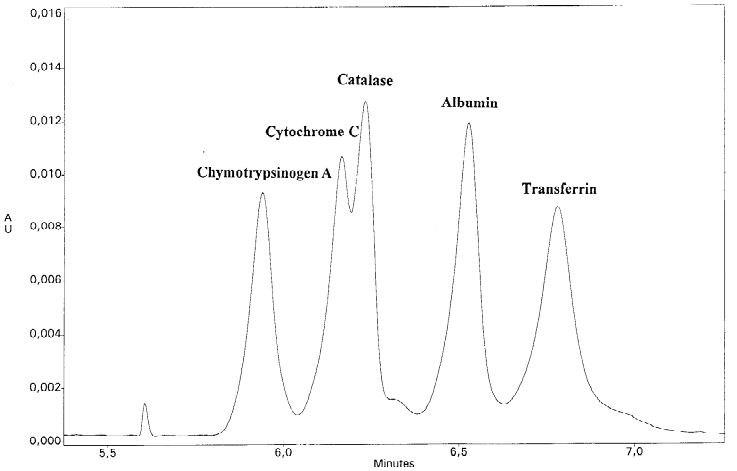

Fig. 2. Standard capillary electrophoresis of model proteins in the presence of SDS in phosphate buffer (50 m*M* NaH_2PO_4, 2 m*M* SDS, pH 2.5), at 10 kV, 25 °C. Samples were dissolved in Milli-Q water made 10 m*M* with respect to SDS. Negative polarity at the inlet. UV detection at 214 nm.

ase, albumin and transferrin. Because of the well known fact that the amount of bound SDS is nearly constant ranging between 90 and 100% (w/w) in proteins possessing –S–S– bonds [23] (for a review see Ref. [24]), one would expect simple reversal in the elution order of individual test proteins, though the selectivity may be affected. As shown in Fig. 2, chymotrypsinogen A eluted first and catalase moved ahead of albumin as expected, but the elution of transferrin was unexpectedly long while the elution of cytochrome *c* occurred much faster (relatively to other members of the mixture) than expected. This may be ascribed to the fact that the position of individual proteins on the electropherogram may in part reflect the affinity of individual solutes to the surfactant used under the conditions selected; the complexation of individual proteins need not necessarily be complete. The other aspect to be considered is that, measured by the w/w protein surfactant ratio, the amount of SDS bound at equilibrium may be as high as 140%, particularly with proteins devoid of –S–S– bonds. (The irregularities in the behavior of glycosidic residues containing proteins have been generally known for years [24]). It must also to be mentioned that the separation of cytochrome *c* and catalase was incomplete.

In order to be sure that our test proteins bound as much of the surfactant as possible, we conducted some experiments in which the samples were prepared in water containing a considerably higher concentration of SDS (50 m*M*) and a typical result is shown in Fig. 3. It appears that using this way of sample preparation the proportion of bound SDS was higher as the separation was consistently ~1 min faster; on the other hand the charge differences between individual analytes were less distinct than in the previous case (sample prepared with 10 m*M* SDS) and the separation of individual components of the test mixture was only indicated. The idea about the differences in the amount of bound SDS is further supported by the interchange in the elution sequence between catalase and cytochrome *c*. In our previously published results [25,26] we also demonstrated unique separation features of systems with SDS high content at low pH. The currently presented results are in good agreement with data published already.

If the conditions used for the separation shown in Fig. 2 for the standard capillary electrophoresis system are applied to the chip separation the result looks like that shown in Fig. 4 (negative polarity at the inlet, conductance detection). Though the separation is rather slow for a chip-based system, the resolution is better than that obtained with a standard

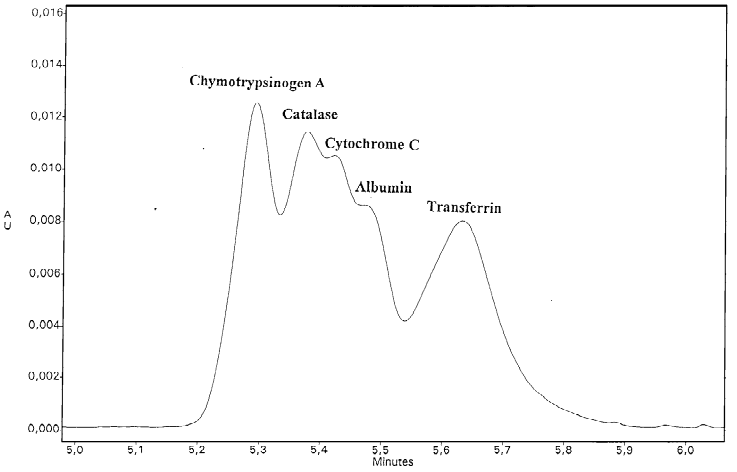

Fig. 3. Standard capillary electrophoresis of model proteins in the presence of SDS in phosphate buffer (50 m*M* NaH_2PO_4, 50 m*M* SDS, pH 2.5) at 10 kV, 25 °C. Samples were dissolved in Milli-Q water containing 50 m*M* SDS. UV detection at 214 nm.

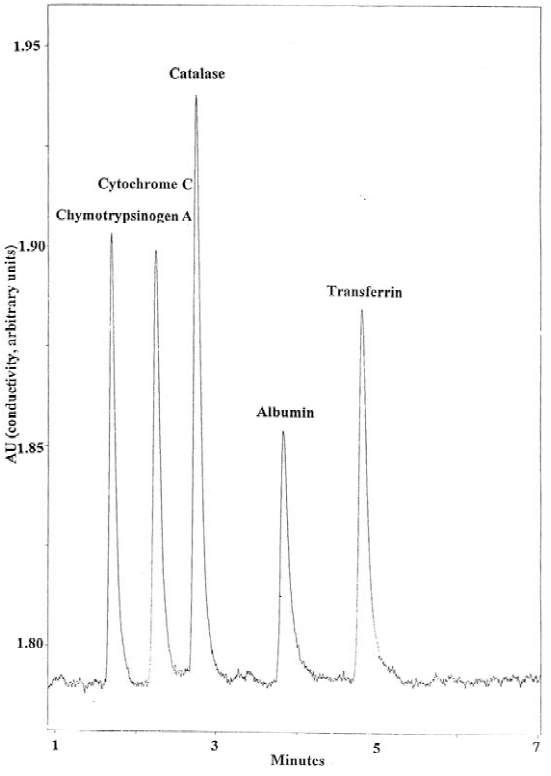

Fig. 4. Chip electrophoresis of the standard protein mixture. 500 V per chip, negative polarity, phosphate buffer (50 m*M* NaH_2PO_4, 2 m*M* SDS, pH 2.5), no thermostatting was used. Samples were dissolved in Milli-Q water made 10 m*M* with respect to SDS. Conductivity detection.

capillary; no change in the elution order of individual proteins was observed on comparison between the standard capillary and chip operational mode.

Using a higher concentration of SDS for sample preparation (50 m*M*) the separation shown in Fig. 5 was obtained. The resolution appears considerably better than that obtained with the standard (capillary) operational mode; the interchange between the elution order of catalase and cytochrome *c* observed with the standard electrophoresis approach was preserved.

4. Conclusions

Using a model set of proteins (cytochrome *c*, albumin, catalase, transferrin and chymotrypsinogen A) conditions for converting these analytes into SDS complexes and their separation by conventional capillary electrophoresis in the negative polarity mode were determined. The best results were obtained when the aqueous solutions of sample compounds were converted to SDS complexes by making the solution 10 m*M* with respect to the surfactant and running the samples prepared in this way in 50 m*M* phosphate, pH 2.5, containing 2 m*M* SDS. The same procedure appeared applicable for chip separations using conductivity detection.

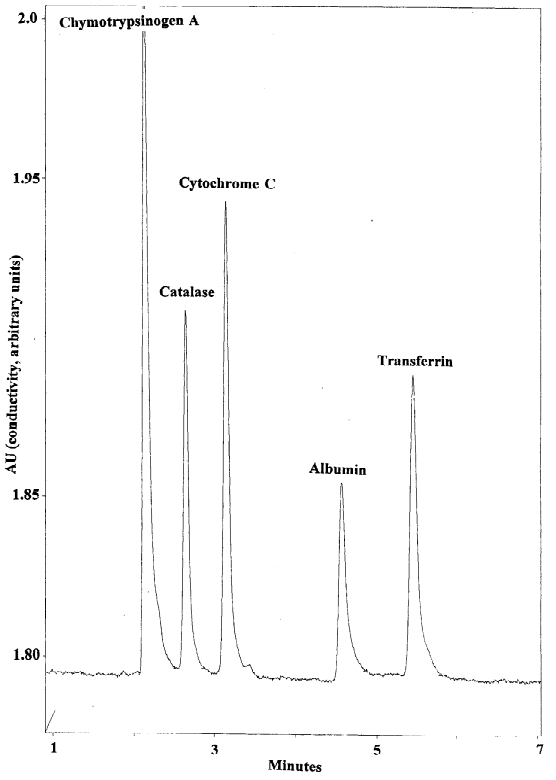

Fig. 5. Chip electrophoresis of the standard protein mixture as in Fig. 4 except that the sample was prepared in 50 m*M* aqueous SDS.

By increasing the concentration of the surfactant during the sample preparation phase, individual proteins attain more negative charge, which consequently leads to altered sequence of their elution. In standard capillary electrophoresis the final resolution seen on the electropherogram is poorer than when the lower concentration of the surfactant (10 m*M*) is used in the preparatory stage. In contrast, no matter which surfactant concentration was used, chip electrophoresis offered better results compared to the conventional set-up. Further investigation on the properties of corundum-based chips for protein separation is in progress.

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