Micropreparation of peptides by capillary electrophoresis for matrix assisted laser desorption mass spectrometry

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Abstract In the separation of peptides by capillary electrophoresis and analysis by matrix assisted laser desorption mass spectrometry, strong suppression of the mass spectrometric signals is a problem with many common electrolytes used in the separation step such as sodium phosphate. We describe an approach employing individual electrolytes selected for highest performance in each process. Suppression with samples collected into phosphate buffers is avoided when citrate, trifluoroacetic acid or hydrochloric acid is used for collection, while phosphate still provides excellent resolution in the capillary. Low concentrations of hydrochloric acid added to the sample/matrix mixture generate essentially adduct-free mass spectra with better signal-to-noise ratios and detection limits (fmol range) than those obtained with citrate or trifluoroacetic acid. Addition of 0.25% ethylene glycol to both the phosphate electrolyte and the sample improves peak shape and resolution, and is crucial for preparative separations in large diameter capillaries.

Key words: Capillary electrophoresis; Matrix assisted laser desorption mass spectrometry; Peptide; Micropreparation; Hydrochloric acid; Trifluoroacetic acid

1. Introduction

Capillary electrophoresis provides high resolution of polypeptides at a sensitive scale (fmol to amol). Separation times are short (5–20 min) and the methodology is applicable over a broad pH range. Although these criteria make capillary electrophoresis of interest in preparation of peptides and proteins for structural analysis, the small loading volumes (nl) and amounts have been a difficulty for preparative work. In recent years, several approaches have been suggested to overcome the limitations. Reports on successful characterization by Edman degradation, amino acid analysis, matrix assisted laser desorption ionization (MALDI) and electrospray ionization (ESI) mass spectrometry of components isolated by capillary electrophoresis have been presented. Protocols include preparation by electrophoresis into sampling vials [1–3], collection onto membrane supports [4,5], direct deposition onto MALDI targets [6,7], collection in capillaries [8,9] or by direct coupling to an ESI source [10–12].

In most preparative applications, the same electrolyte is used for both the separation in the capillary and the sampling process. This can be a significant disadvantage if the electrolyte has low compatibility with the technique for structural analysis. This is particularly true in MALDI applications, when phosphate buffers are used for separation and collection. While phosphate provides excellent resolution in capillary electrophoresis [1–3,7,13,14], it gives a strong signal suppression during laser desorption, which adversely affects the detection limit of the system.

We now present an approach for sample collection and MALDI analysis of peptides separated by capillary electrophoresis. It includes selection of the most suitable and compatible electrolyte for separation in the capillary and for collection for MALDI, respectively. In this manner, compromise alternatives with average to poor performance characteristics are avoided.

2. Materials and methods

2.1. Materials

Naturally occurring peptides (Table 1) were synthesized (SA-1, SA-

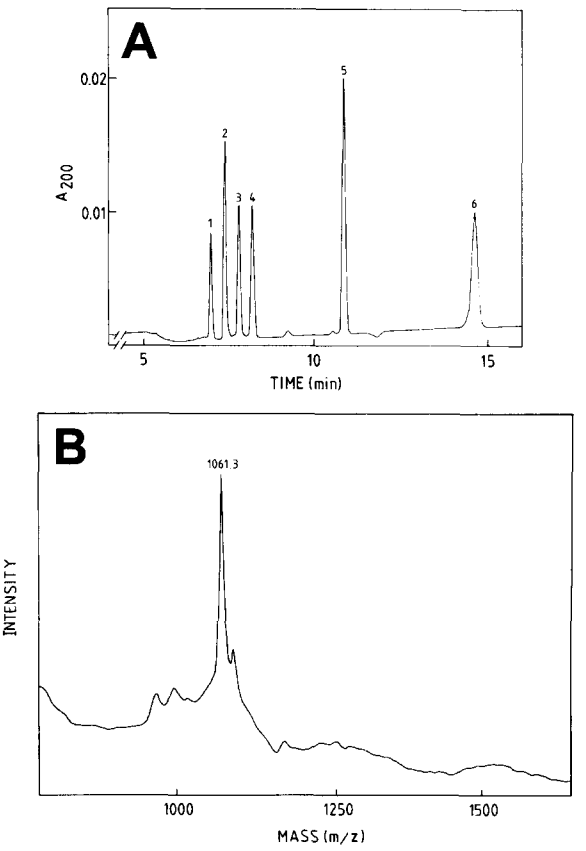

Fig. 1. (A) Capillary electrophoresis of peptides (cf. Table 1) in amounts 1–2 pmol and sizes 5–17 residues (50 mM phosphate buffer, pH 2.5, 75 μm, 20 kV). The component corresponding to peak 2 was collected in a single run into 20 mM citrate buffer, pH 2.5, and a part of the preparation (200 fmol) was analyzed by MALDI mass spectrometry (B). The mass detected 1061.3 (MH^+) identifies bradykinin (MH^+ 1061.2).

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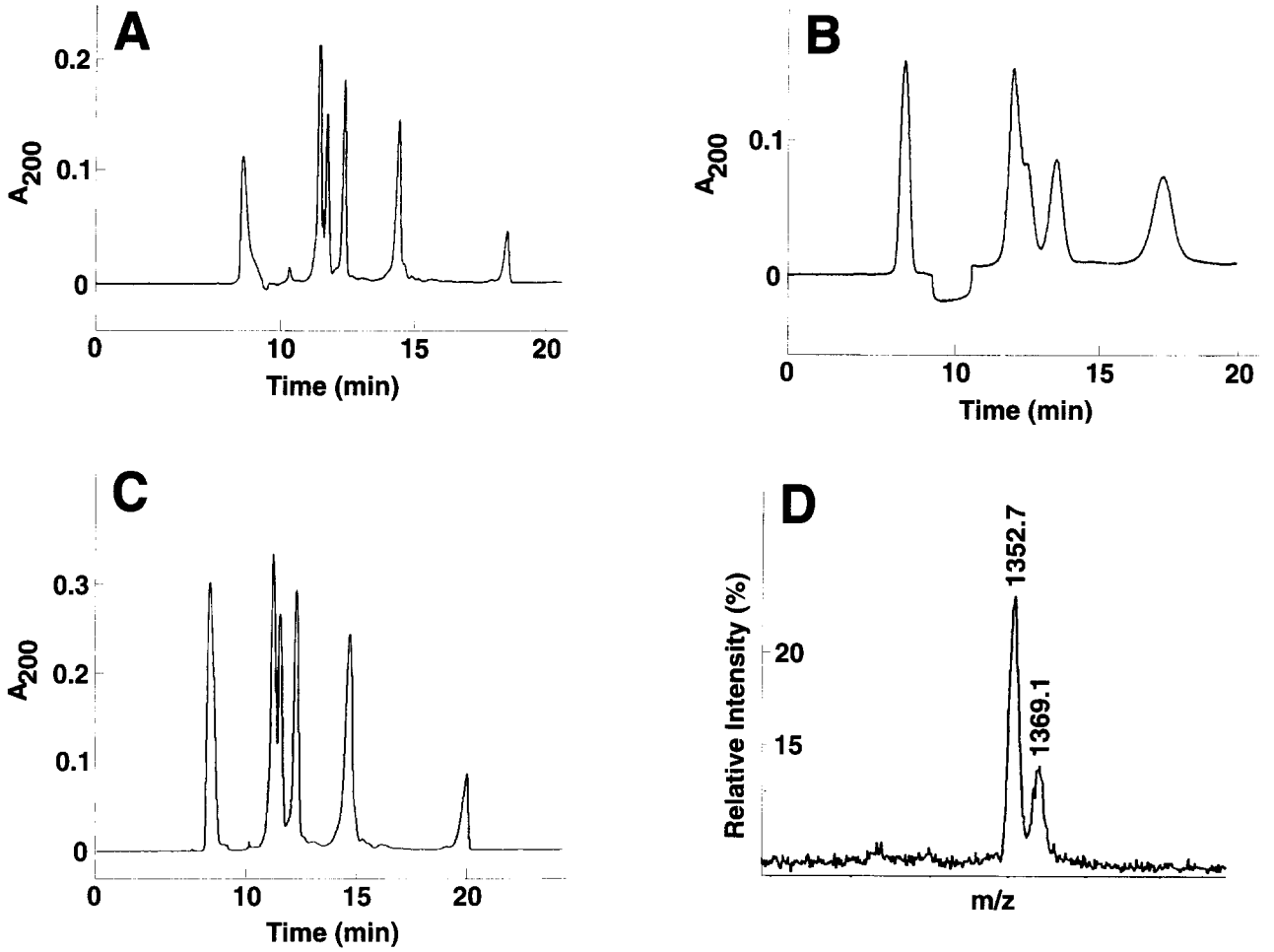

Fig. 2. (A–C) Capillary electrophoresis of peptides (cf. Table 1) in 50 mM phosphate buffer, pH 7.0, containing 0.25% ethylene glycol (150 μm, 20 kV). (A,C) With and (B) without the addition of ethylene glycol (0.25%) to the sample. In (A,B), 3–6 pmol was injected, in (C) 6–12 pmol. (D) MALDI mass spectrometry of 0.5 pmol substance P after preparation by a single run according to (C) and collection into 1% TFA supplemented with 0.25% ethylene glycol. The higher mass detected corresponds to the oxidized peptide (methionine to methionine sulfoxide).

2 and C-8) (cf. [15]) or purchased from Sigma (bradykinin, substance P and leucine enkephalin). Peptides from digests were prepared from carboxymethylated yeast alcohol dehydrogenase (Table 1) [16] treated with endoproteinase LysC (Wako Chemicals, Japan) at an enzyme to substrate ratio of 1:40 in 100 mM ammonium bicarbonate (4 h at 37°C).

Table 1
Data for peptides and proteolytic fragments analyzed by capillary electrophoresis and MALDI mass spectrometry

Peptide	Mass	Structure
1[a] SA-2	1261.3	EPSHWKHVEL
2 Bradykinin	1060.2	RPPGFSPFR
3 SA-1	1176.0	PPQTEHTEHT
4 Substance P	1347.6	RPKPQQFFGLM
5 Leucine enkephalin	555.6	YGGFL
6 C-8	1597.7	ELGGGPGAGDLQTLALE
Alcohol dehydrogenase (yeast) LysC-digest, fragments:		
8–17[b]	1136.3	GVIFYESHGK
39–59	2477.7	YSGVC[c]HTDLHAWHGDWP LPTK
335–347	1407.6	GQVVGRYVVDTSK

[a]Numbers 1–6 refer to the migration order in capillary electrophoresis, cf. Fig. 1.
[b]Residue numbers [16].
[c]Carboxymethylated (native cysteine mass increased by 58.04 Da).

2.2. Capillary electrophoresis

Separations were carried out with a Beckman P/ACE 2100 capillary electrophoresis instrument. Fused silica capillaries with inner diameters of 75 or 150 μm, and total lengths of 57 cm, were from Polymicro Technologies (Phoenix, AZ). Injections were performed by pressure and the UV detector (200 nm) was positioned 7 cm from the outlet end. The electrolytes used in the capillaries for separation of sample components were 50 mM sodium phosphate buffer, pH 2.5 or 7.0, the latter supplemented with 0.25% ethylene glycol. Isolation of components for mass spectrometry was carried out by a programmed positioning at the outlet end of conically shaped vials (Beckman) filled with an electrolyte (10–15 μl) suitable for subsequent MALDI analysis. The collection buffers tested were 1% trifluoroacetic acid (TFA), with or without 0.25% (v/v) ethylene glycol, 20 mM sodium citrate buffer, pH 2.5, and 25 mM HCl.

2.3. MALDI mass spectrometry

Mass determinations were carried out with a Finnigan MAT Lasermat 2000 instrument. A 10 mg/ml solution of α-cyano-4-hydroxycinnamic acid (Sigma) in aqueous 70% acetonitrile was employed as matrix. Sample solution and matrix (0.5 μl each) were premixed before application onto a stainless-steel target. Acidification with 0.1% TFA was tested for some samples collected in 20 mM citrate. The droplet was dried by shortly placing the target on a heating block

Table 2
Signal-to-noise ratios recorded during laser desorption of 1 pmol of a 9 residue peptide (bradykinin) after addition of the indicated buffers and acids to the sample/matrix mixture

Addition	Relative signal-to-noise ratio
1% trifluoroacetic acid	0.9
5% trifluoroacetic acid	0.9
20 mM sodium citrate	1
0.025 M HCl	2.6
0.05 M HCl	2.5
0.1 M HCl	1.2
15% formic acid	0.1
15% acetic acid	0.1

Values are based on averages of 3–4 determinations at different laser aims and are normalized to the value of 1 for citrate.

before introduction into the mass spectrometer. Mass determinations were made using the external calibration of the instrument.

3. Results and discussion

To avoid signal suppression in MALDI of peptides prepared by capillary electrophoresis and still be able to use phosphate buffers for separation, we have tried addition of MALDI-compatible electrolytes in the collection vial, while maintaining optimal phosphate conditions in the capillary. Sodium citrate buffer at low concentration and pH (5–10 mM, pH 2.5) has been employed for separation and collection of samples for MALDI analysis with only minor suppression [7,17]. We have now tested citrate for collection only, and at a higher concentration (20 mM) for better stabilization of the current during electrophoresis to the sampling vial. This proves to be efficient, combining good separation and reproducible collection for sensitive mass analysis (Fig. 1). We also notice that addition of 0.1% TFA to the sample/matrix mixture for samples collected into 20 mM citrate, gives a better signal-to-noise ratio and peak separation improving the detection limit about 3-fold.

A problem with fused silica capillaries is sample adsorption to the negatively charged wall with its ionized silanol groups which may result in broad peaks, loss of resolution and a 'memory effect' (carry over) [14,18]. To overcome these difficulties, several approaches have been suggested, including coating of the capillary wall [7,19–22] and use of additives to the electrolyte (cf. [18]). Addition of ethylene glycol to samples (20–30% (v/v)) improves resolution of polypeptides in capillary electrophoresis [2,23]. However, a drawback is the broad UV absorption of ethylene glycol resulting in a high background and poor detectability. We have therefore tested low concentrations of ethylene glycol (0.25% (v/v) found optimal) both in the sample and in the electrolyte (50 mM phosphate buffer, pH 7.0), which results in improved peak shape and better resolution (Fig. 2A,B). The low concentration of ethylene glycol makes it possible to use detection at 200 nm, and the neutral pH is an advantage when biological activity must be preserved. To increase the capacity and loading, a large inner diameter, 150 μm instead of the standard 75 μm, was tested for preparation of samples for MALDI analysis. Negative effects such as increased separation time, band broadening and Joule heating were similarly reduced if 0.25% ethylene glycol was added to both the electrolyte (50 mM phosphate, pH 7.0) and the sample (Fig. 2C). Preparation using this system and electrophoresis into 1% TFA (see below) containing 0.25% ethylene glycol was tested revealing essentially sodium adduct free mass spectra (Fig. 2D).

To survey the possibilities to improve further the detectability in MALDI analysis of peptides prepared by capillary electrophoresis, we tested acidification with TFA, citrate, HCl, formic and acetic acid (Table 2) directly on the target plate of the sample/matrix mixture. In relation to formic and acetic acid, commonly employed for this purpose, a significantly increased signal-to-noise ratio is observed with TFA, citrate and HCl (Table 2). The peak shapes and accuracies are markedly better with TFA and HCl due to the much smaller or even absent sodium adducts. These results prompted us to try TFA directly in the collection vial. Capillary electrophoresis using 50 mM phosphate buffer, pH 2.5 or 7.0 (cf. Fig. 2D), and collection into 1% TFA reveals stable current conditions and reproducible recovery of peptide components. The variation in migration time is less than 1%, as analyzed for up to 15 consecutive preparative runs. Collected samples are directly mixed with matrix solution without the need for extra acidification and the detectability in MALDI mass spectrometry is comparable or better than when citrate buffer is used (Fig. 3). We conclude that direct collection into aqueous TFA results in better MALDI signal-to-noise ratios and better separation of sodium adduct peaks than collection into sodium citrate [17] or ammonium phosphate [6,14]. The survey of the effects of acidification (Table 2) shows very good data for HCl at concentrations below 0.1 M. The signal-to-noise ratio is increased by a factor larger than 2 versus that obtained for citrate and TFA. This makes 25 mM HCl (Table 2) a highly

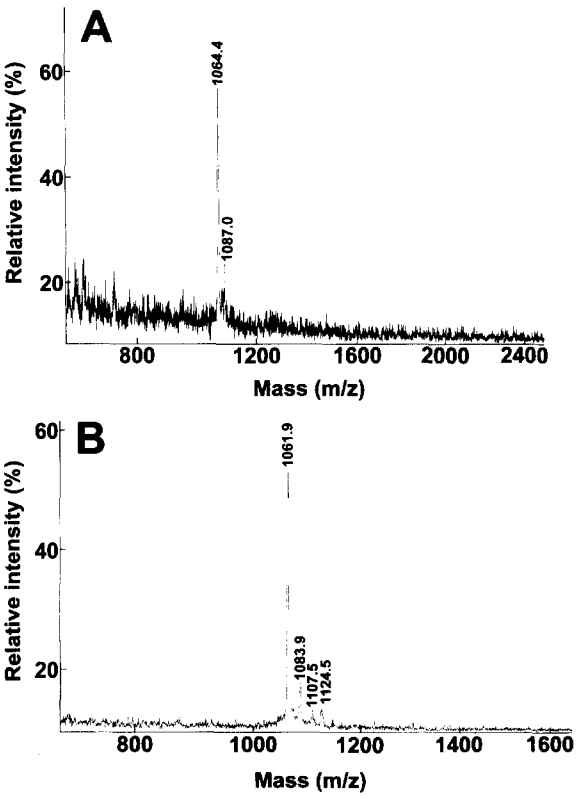

Fig. 3. MALDI mass spectrometry of bradykinin after separation by capillary electrophoresis in phosphate buffer (cf. Fig. 1) and collection into 20 mM citrate buffer, pH 2.5 (A) and into 1% TFA (B). The higher masses detected correspond to sodium adducts.

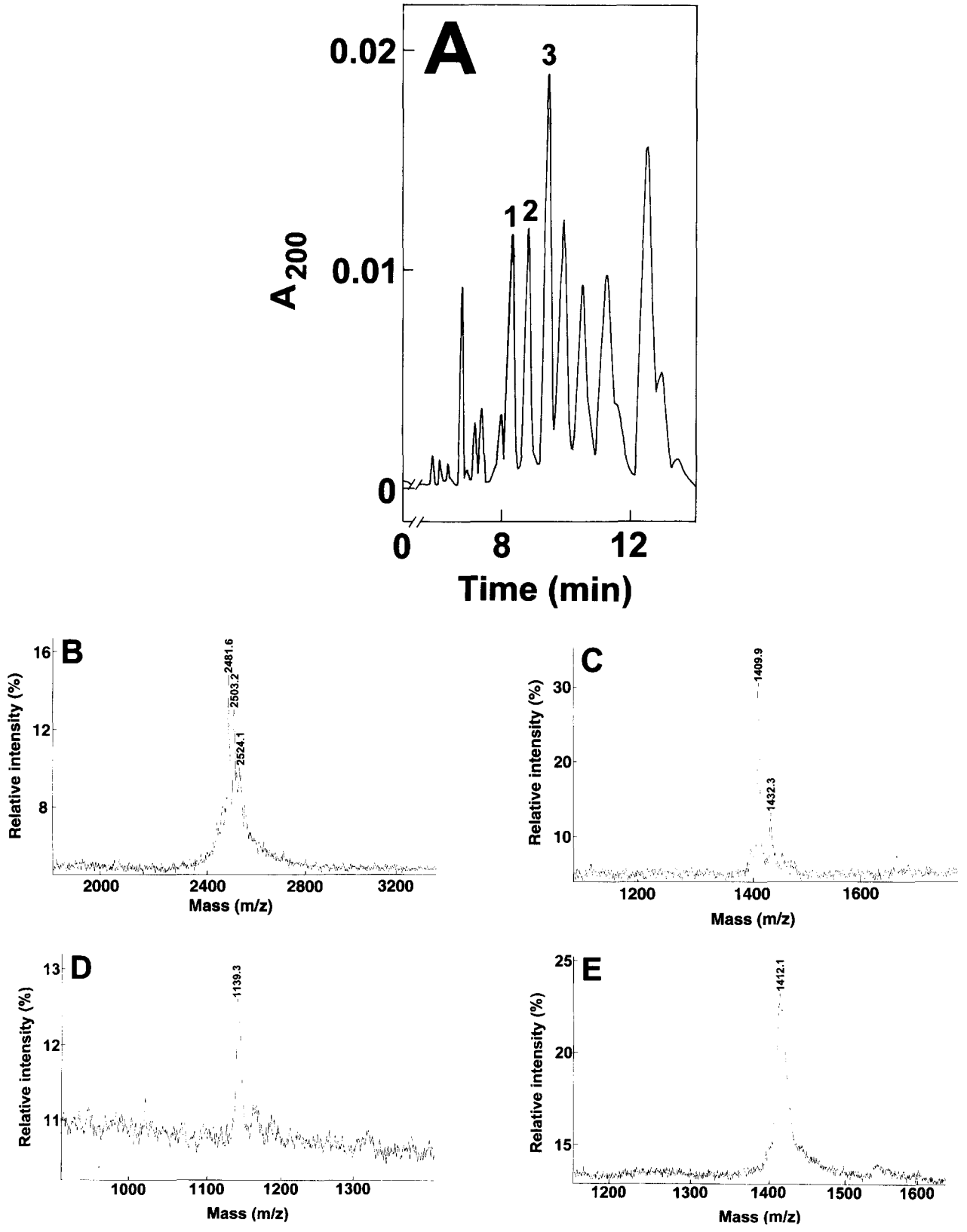

Fig. 4. (A) Capillary electrophoresis (50 mM phosphate buffer, pH 2.5, 75 μm, 20 kV) of 1 pmol of a LysC-specific digest of yeast alcohol dehydrogenase [16]. Fragments were collected for MALDI mass spectrometry into 20 mM citrate buffer, pH 2.5 (B,C) or into 25 mM HCl (D,E). MALDI analysis of the components corresponding to peaks 1–3 in (A) identifies: (B) fragment 39–59 (peak 2), (C) fragment 335–347 (peak 3), (D) fragment 8–17 (peak 1) and (E) fragment 335–347 (peak 3) now collected to HCl. For structures, cf. <TBLR>1</TBLR>. The higher masses detected in B and C correspond to sodium adducts.

promising candidate for collection of polypeptides for MALDI mass spectrometry.

We have also tested preparation of protein fragments from LysC digests of yeast alcohol dehydrogenase [16], employing either 20 mM citrate buffer, pH 2.5, or 25 mM HCl in the collection vial (Fig. 4). Subsequent MALDI analysis identifies the fragments and localizes them in the primary structure [16] (Fig. 4B–E and Table 1). Notably, 25 mM HCl generates spectra free from sodium adducts and giving strong and clear signals for unambiguous fragment identification (Fig. 4D,E, and Table 1).

In conclusion, the results show that a careful choice of electrolyte for separation and collection, respectively, is of great importance for compatibility with the subsequent structural analysis. Use of phosphate buffer in the capillary and aqueous TFA or dilute hydrochloric acid in the collection vial, constitutes a practical and efficient approach for isolation of peptides for MALDI mass spectrometry.

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