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Short communication

Analysis of non-covalent protein complexes by capillary electrophoresis–time-of-flight mass spectrometry

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Abstract

A capillary electrophoresis–electrospray ionisation time-of-flight mass spectrometry (CE–ESI-TOF-MS) method for characterisation of non-covalent protein complexes is described using a coaxial liquid sheath-flow sprayer. The CE capillary was connected to the mass spectrometer using a commercial CE–MS sprayer mounted on a ceramic holder of the ESI interface of the mass spectrometer. Using myoglobin (Mb) as an example of non-covalent protein complex, the effect on complex stability caused by organic modifiers added to the sheath liquid was analysed. Depending on the amount of methanol, either intact Mb or the apoprotein and the prosthetic heme group were detected.

Keywords: Coaxial liquid sheath flow; Mass spectrometry; Proteins

1. Introduction

Proteins play key roles in a variety of biological processes by forming specific non-covalent complexes with other proteins, nucleic acids or small ligands, such as metal ions, drugs or nucleotide co-factors.

The common techniques for obtaining structural information of such non-covalent interactions are X-ray crystallography, nuclear magnetic resonance spectroscopy (NMR) or circular dichroism (CD) [1]. However, in many cases these methods are time consuming and larger amounts of protein are required.

Since the emergence of electrospray ionisation (ESI) by Fenn et al. [2] and notably, the implementation of nano electrospray ionisation (nanoESI) by Wilm and Mann [3], mass spectrometry has also become a powerful tool for the study of biomolecular non-covalent interactions [4,5]. Due to its gentle nature of ionisation yielding nearly no molecular fragmentation, ESI allows the detection of intact, weakly bound complexes. Particularly, in conjuction with high-resolution mass spectrometry nanoESI appears to be a well suited technology for investigations of non-covalent protein complexes containing ligands of low molecular mass.

In the last years, capillary electrophoresis (CE) has matured to a valuable alternative or complementary technique to HPLC due to short analysis time, low sample consumption, high separation efficiency, and relatively low operating costs [6]. The versatility of this technique makes it suitable for separations of chemically diverse substances, particularly for peptide and protein separation.

In addition, in the last years the combination of

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CE and mass spectrometry (MS) has become a promising analytical tool, hyphenating high-performance separation technology and high-performance detector technology [7]. Hence, a number of different CE–MS approaches have been explored and resulted in the development of different CE electrospray interfaces [7,8].

In this article the hyphenation of CE to high-resolution time-of-flight (TOF) MS using a sheath flow interface was described allowing the detection of non-covalent protein complexes. First, results demonstrating the effect of organic modifier added to the sheath liquid on complex stability are presented.

2. Experimental

2.1. Chemicals

Methanol SupraSolv was purchased from Merck (Darmstadt, Germany). Myoglobin (Mb), apomyoglobin (apoMb) from horse skeletal muscle, aqueous ammonia solution, acetic acid and ammonium acetate were obtained from Sigma (Taufkirchen, Germany) and used without further purification.

CE running buffer was prepared by dissolving 50 m*M* ammonium acetate in deionized water adjusted to pH 8.3 with a 12.5% aqueous ammonia solution.

Standard solutions of Mb and apoMb were prepared in CE running buffer at a final concentration of 1 $\mu g\ \mu l^{-1}$ (1.7 pmol loaded on the CE capillary).

2.2. Capillary electrophoresis

CE separations were performed on a $HP^{3D}CE$ capillary electrophoresis system from Agilent Technologies (Waldbronn, Germany) using HP ChemStation software. Samples were loaded by hydrodynamic mode at 50 mbar for 30 s. Separations were performed on a non-coated fused-silica capillary of 80 cm×360 μm O.D.×50 μm I.D. (CS Chromatographie Service, Langerwehe, Germany). A constant voltage of 30 kV was impressed throughout all analyses (depending on the samples being analysed the current varied between 28 and 34 μA). The capillary was thermostated at 25 °C. Prior to analysis the capillary was flushed with 1 *M* NaOH for 10 min, then rinsed with water for the same time and finally conditioned with CE running buffer for 10 min.

2.3. Mass spectrometry

Mass spectrometry was performed on a Mariner orthogonal time-of-flight mass spectrometer (Applied Biosystems, Foster City, CA, USA) operating in the reflectron mode. Instrument controlling and data acquisition were performed using the Mariner instrument control panel V 4.0.0.0 and the data explorer V 4.0.0.1 (Applied Biosystems).

2.3.1. CE–MS coupling

The CE capillary was connected to the mass spectrometer using a commercial CE–MS sprayer (Agilent Technologies) mounted on a ceramic holder in the ESI interface of the mass spectrometer. Sheath

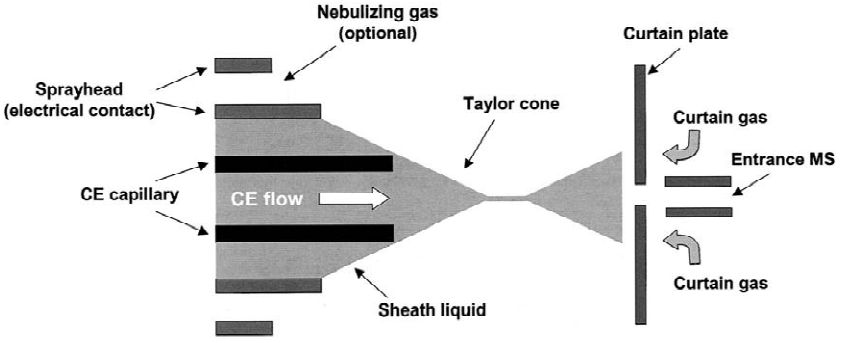


Fig. 1. Scheme of the coaxial sheath flow interface used in the CE–TOF-MS experiments.

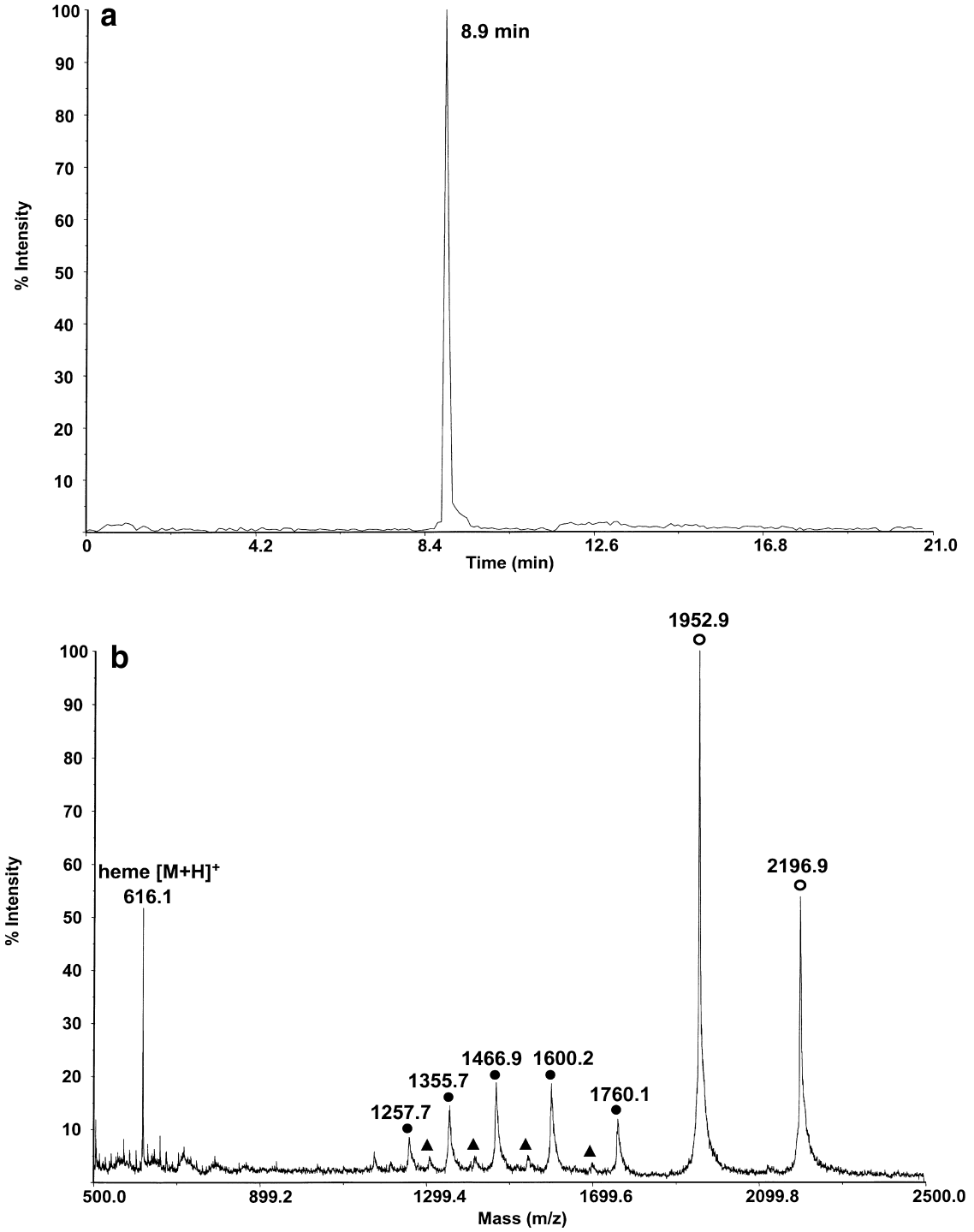


Fig. 2. Results from CE–TOF-MS experiments of myoglobin (Mb) under non-denaturating conditions (running buffer 50 m*M* ammonium acetate pH 8.3, sheath liquid 10 m*M* ammonium acetate pH 6.8–methanol, 50:50, v/v); details are given in the Experimental section. (A) Extracted ion electropherogram (XIE) of Mb (1.7 pmol injected); (B) extracted mass spectrum of peak at 8.9 min. Charge-state distributions of Mb (○ and ●) and apoMb (▲). (C) Deconvoluted mass spectrum of (B) indicates intact Mb (M_r~17 567) and to a lower abundance apoMb (M_r~16 952).

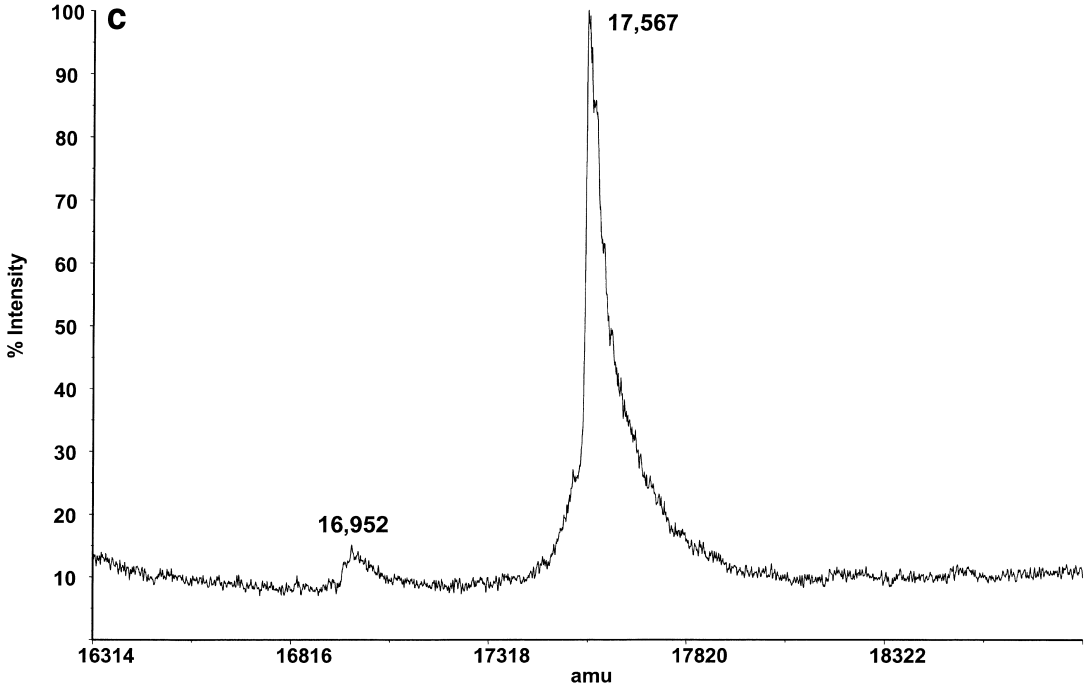


Fig. 2. (*continued*)

liquid consisted of 10 m*M* ammonium acetate in water pH 6.8–methanol (50:50, v/v) and was delivered using a syringe infusion pump (Harvard Apparatus, South Natick, MA, USA) at flow rates of 0.7 μl min^{-1}. Additional nebuliser gas (''sheath gas'') was not used.

2.3.2. Nano electrospray ionisation

For nanoESI the mass spectrometer was equipped with a nanospray source operating in the positive ionisation mode. Nanospray capillaries of borosilicate glass coated with gold–palladium (Protana, Odense, Denmark) were filled with 10–15 μl of standard protein solution. A video camera was used to monitor the cutting of the capillary tip at the curtain plate and its positioning. The distance between capillary and curtain plate was about 1–2 mm. To initiate the nanospray-process, a syringe filled with air was used to induce some back pressure.

2.3.3. Operating conditions of the mass spectrometer

For nanoESI-MS the spray tip voltage was set to 1650 V. For CE–MS experiments no additional tip voltage was applied. The temperature of the nozzle and the focusing quadrupole [only radiofrequency (RF) voltage applied for ion transmission] were both set to 90 °C. Curtain gas (nitrogen) was set to a flow of 1 l min^{-1}. Spectra were acquired in the mass range from 500 to 2500 amu at a scan rate of 5 s per spectrum. The mass axis was externally calibrated using a standard peptide mixture of reserpine and neurotensin (Applied Biosystems, Foster City, CA, USA), each 1 μg ml^{-1}. Additional internal calibration was achieved by using charge state distributions of apoMb.

3. Results

Our experimental strategy involved CE separation of the non-covalent protein complexes followed by TOF-MS. Myoglobin with the non-covalent bound heme group was chosen as a suitable candidate to validate this strategy.

To couple CE and TOF-MS the mass spectrometer

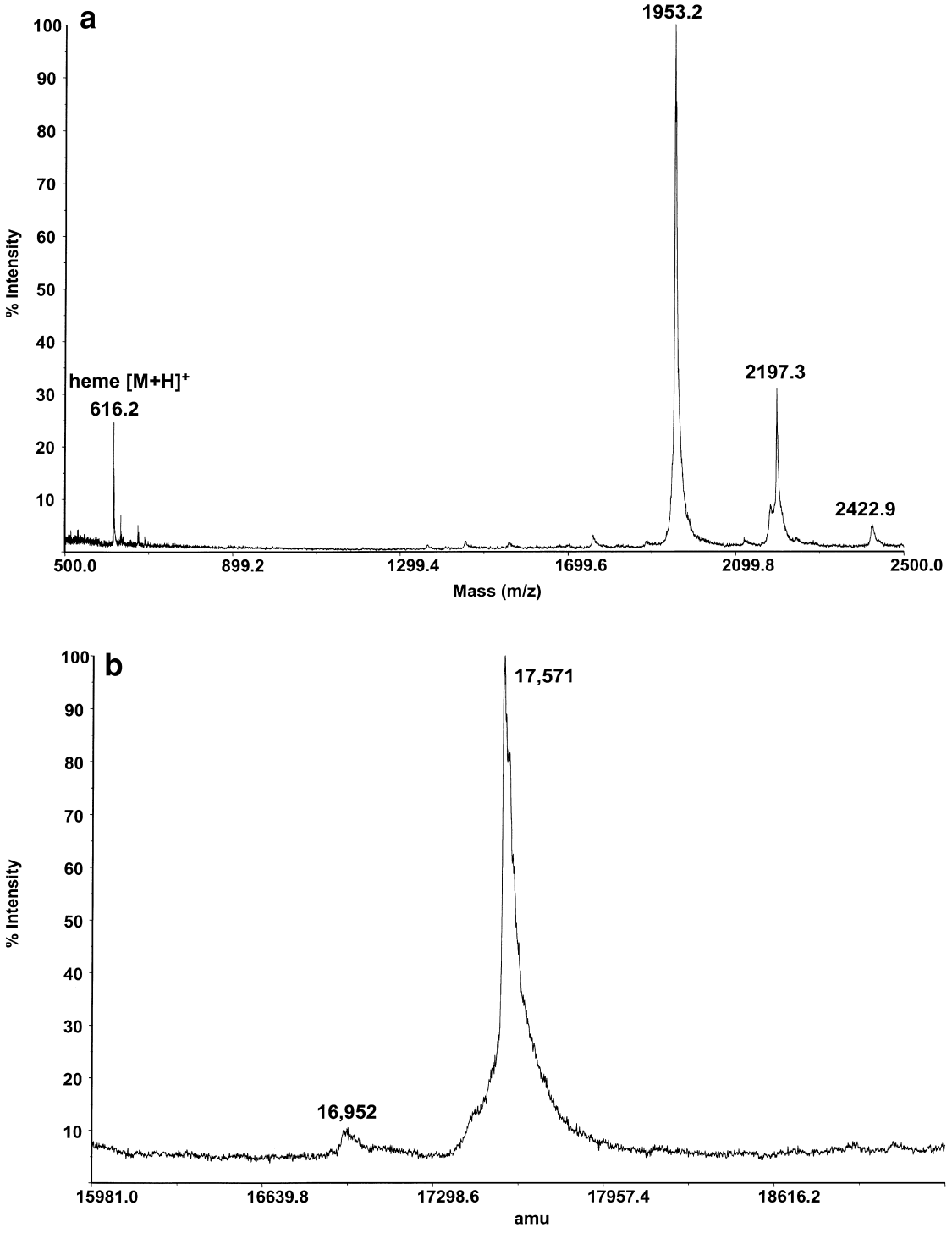
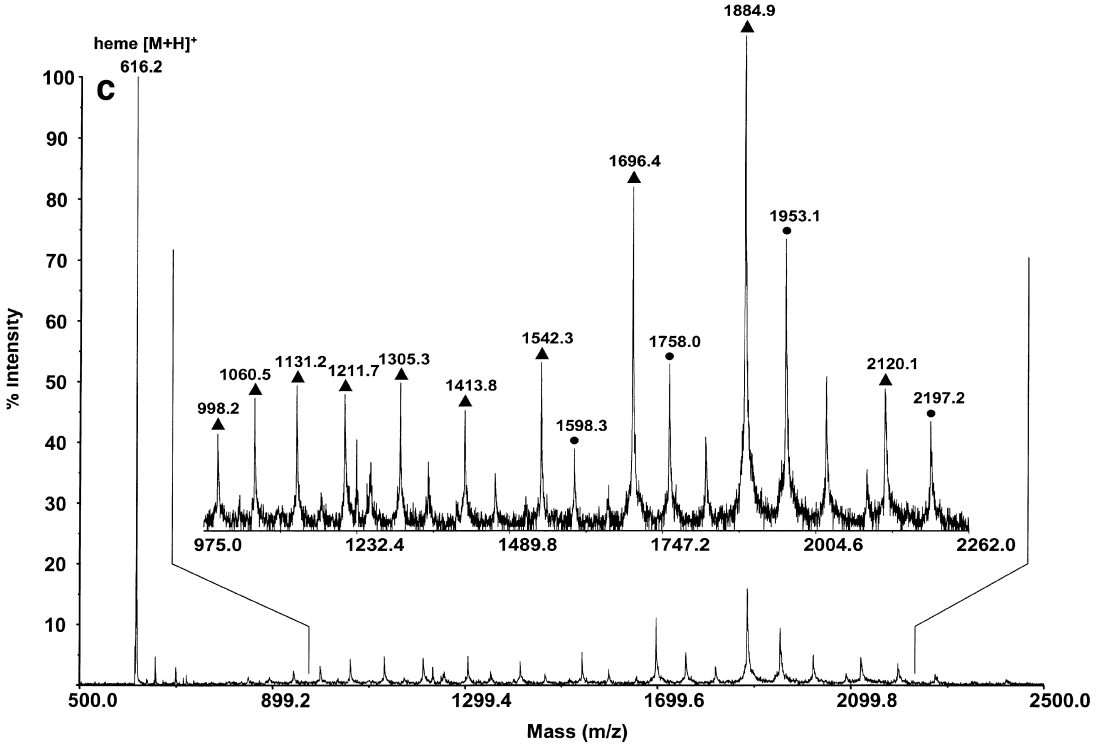


Fig. 3. NanoESI mass spectra of Mb. (A) under non-denaturating conditions (5 m*M* ammonium acetate pH 6.8. (B) Deconvoluted mass spectrum of (A). (C) Addition of 50% methanol; ▲ indicates charge-state distribution of apoMb, ● indicates charge-state distribution of Mb; (D) deconvoluted mass spectrum of (C).



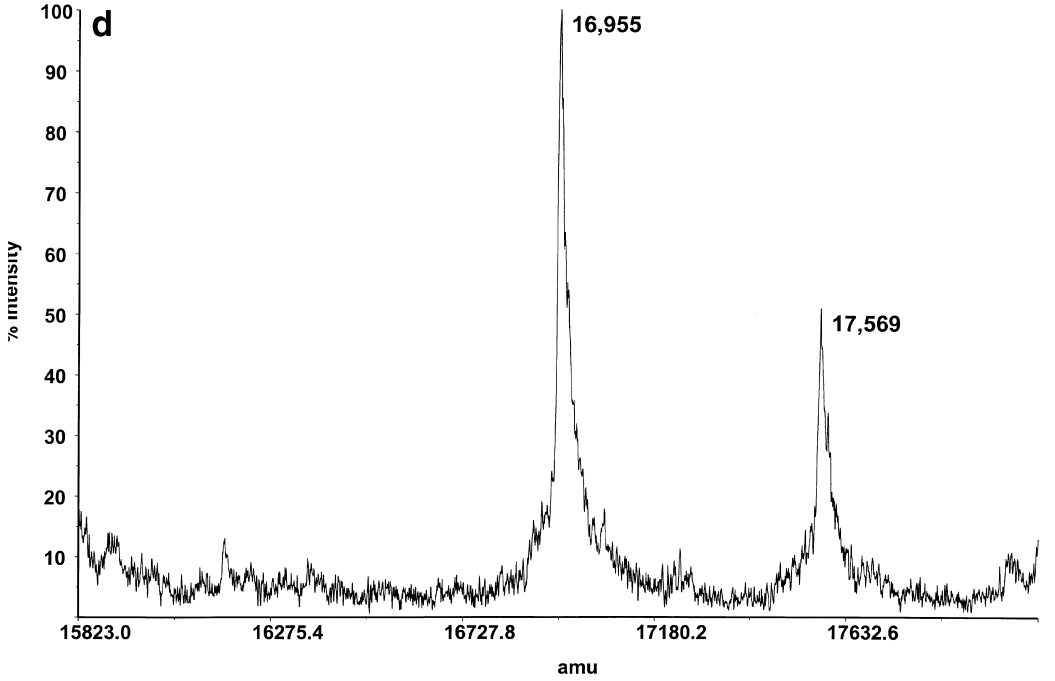


Fig. 3. (*continued*)

was equipped with an electrospray ionisation source with a sheath flow system. The main advantage of a sheath flow interface is that it is easy to use due to its simple and reproducible construction. By running the CE–MS sprayer without sheath gas and at a reduced sheath flow, the disruption of the electrospray was avoided. Fig. 1 shows the scheme of the CE–MS sprayer used in the experiments. Additional spray tip potential controlled by the mass spectrometer was not impressed. The applied CE voltage generated a tip potential (approx. 1000 V) that was sufficient enough for a stable electrospray in the CE–MS mode.

Sheath liquids consisting of aqueous ammonium acetate solution with methanol as organic modifier were found to exhibit the best results for stable electrospray. The results of CE–TOF-MS analysis of Mb are illustrated in Fig. 2.

The electropherogram (Fig. 2A) shows that the migration of Mb from the capillary was completed within 10 min. The peak at 8.9 min led to the extracted mass spectrum shown in Fig. 2B. It exhibits two charge-state distributions of Mb containing the prosthetic heme group. These two groups of charge-states are attributed to a more ‘‘native-like’’ (○) and a more unfolded (●) conformation of Mb, respectively. The signal labelled with ▲ and the signal at m/z 616.1 (heme group) indicates the partial dissociation of the Mb complex. This was clearly revealed by the corresponding mass deconvolution (Fig. 2C) showing a dominant signal for Mb and at a lower signal intensity for apoMb. Probably, one reason for partial dissociation of Mb was the amount of methanol added to the sheath liquid, since methanol is known to affect protein conformation.

In order to evaluate the mass spectra achieved from these CE–MS runs, additional nanoESI experiments were carried out. The results ot these experiments regarding the effect of methanol on complex stability are illustrated in Fig. 3. Whereas non-denaturating conditions (without methanol) led to mass spectra of intact Mb (Fig. 3A), increasing amounts of methanol caused the dissociation into apoprotein and heme group (Fig. 3C). These findings were confirmed by mass deconvolution (Fig. 3B,D).

4. Concluding remarks

The results described above clearly demonstrate the possibility of detection of non-covalent protein complexes like myoglobin with CE–ESI-TOF-MS. Although easy to use the sheath liquid is a critical factor. Depending on the amount of organic modifiers, either intact complexes or their dissociated products were detected. In addition to these findings regarding organic modifiers, first results show that even slight changes in pH value of the sheath liquid have an influence on the stability of Mb. Therefore, advanced studies are necessary. As a further alternative a sheath-less interface will be tested in the near future.

Acknowledgements

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