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# THE ON-LINE COMBINATION OF CAPILLARY ISOTACHOPHORESIS WITH MASS SPECTROMETRY

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Capillary zone electrophoresis (CZE) has been demonstrated to have tremendous potential for high-resolution separations of ionic compounds of biological interest. The small capillary diameter (<100 μm) used in CZE and the need to keep the ionic strength of the sample two orders of magnitude less than that of the buffer place rather stringent limits on the amount of sample that can be injected. Isotachophoresis in a capillary format (CITP) is a complementary scheme that overcomes these limitations (1). The CITP/MS combination has several attractive features. First, there are no fundamental limits on the sample size that can be introduced. Second, CITP can result in the concentration of samples that are injected in dilute solutions. Third, once the separation is developed the sample elutes continuously, allowing efficient use of detector time. Fourth, the ideal peak shape in CITP is sharp sided and flat topped. This is an ideal peak shape for mass spectrometric detection. The scan speed of most mass spectrometers is not challenged and MS/MS analysis is facilitated (2,3).

Electrospray ionization (ESI) has been shown to be an effective means of ionizing labile biopolymers from solution. In this work we present the results of an examination of two systems using CITP coupled to mass spectrometry by an ESI interface of our design (2).

Two mass spectrometers were used in these studies. The first was a single stage quadrupole. It was assembled from Extrel components and the vacuum system was designed for an atmospheric pressure inlet. An rf only set of rods precedes the analysis stage to aid in efficient transport of ions. The second was a Sciex 6000E triple quadrupole. The instrument was used with our ESI interface. The electrophoresis was done in a 100μm i.d. fused silica capillary column. DB-17 and OV-1 coated columns were used. The effect of treating the inside of the column is to eliminate electroosmosis and reduce peptide or protein binding to the surface. To minimize the total time, once the steady state has been reached the sample was flow migrated from the column by the application of a small pressure head.

An equimolar mixture of the small proteins bradykinin ($M_r$ 1060) and angiotensin ($M_r$ 1296) was prepared from standards obtained from Sigma. The leading buffer was 0.01M ammonium acetate titrated to pH 4.9 with acetic acid. The sample was prepared at $10^{-4}$M each in the leading buffer. The trailing buffer was 0.01M $Ala^+$ titrated with $Ba(OH)_2$ to pH 7.1. The column was 2.5 m long with a DB-17 stationary phase. The column was initially filled with the leading electrolyte and then a sample band 64 cm long was loaded by pressurizing the sample reservoir. The column was transferred to the trailing buffer reservoir and the separation was developed at 58 kV off line for 15 minutes. The electrospray was then started. The sheath was methanol and the sample was eluted with voltage applied under a modest pressure head.

The separation reached steady state with two components separated into their own bands. The presence of a valley between the two components indicated the presence of impurities of intermediate mobility. The single ion chromatograms demonstrated the multiple charging which is so characteristic of ESI. Spectral scans taken over the mass range of interest during the

elution of each of the major components showed clean spectra. A spectral scan taken in the valley between the bands for two components indicated small amounts of poorly characterized impurities of intermediate mobility.

An enzymatic digest of glucagon was prepared as an example of a less well characterized mixture. Since the enzyme was not of high purity the cleavages are not well characterized. It consisted primarily of trypsin and chymotrypsin. Trypsin is highly specific cleaving only Lys- and Arg-bonds. Chymotrypsin is less specific and is in fact a family of enzymes. Thus although a weak digest of glucagon by trypsin should lead to only four products, the presence of other enzymes considerably complicated the possible product mixture.

The leading buffer was 0.01M ammonium acetate titrated to pH 4.6 with acetic acid. The sample was made up in the leading buffer to a concentration of $10^{-4}$M. The trailing electrolyte was 0.01M acetic acid. The column was 0.75 m long with an OV-1 stationary phase. The column was initially filled with the leading electrolyte and then a sample band was loaded hydrostatically. The column was transferred to the terminating electrolyte and the development was started. The ESI was used continuously from the very beginning with a methanol sheath. The voltage was manually ramped to 10 kV over a period of 9 minutes. After 30 minutes the electrolyte reservoir was raised 5 cm to elute the separation from the column. In a separate experiment an on-column UV detector operated at 210 and 280 nM was used to monitor the separation under identical conditions.

Single ion plots for the most prominent peaks seen in the spectra show the bands in the beginning and end of the sample are more fully developed while those in the center still exhibit mixed band characteristics. Most, but not all, of the ions could be correlated with expected digest fragments. As would be expected, the single amino acid residue Arg is present and has the highest mobility among the sample components. The next band to elute is also well characterized and correlates with the T1 (2+) fragment. Two mixed bands correlating with the $T2^{+}$ and T4-478 (2+) fragments appear next. The next two bands are again well developed but do not correlate with any expected major digest fragment. The last band to elute is again well characterized and correlates with the T4-1049 (2+) fragment.

The relatively long period of time over which a peak elutes in CITP makes it an attractive candidate for MS/MS analysis. Such an analysis was done on the leading and trailing bands of the sample. They gave structurally informative results that can be used to confirm the tentative identifications.

The combination of CITP with mass spectrometric detection is a promising but still developing technique. The interface to mass spectrometry does not detract from the separation power of this technique. There is considerable variation in the sensitivity of ESI for the various fragments in a typical digest. Although most of the expected fragments were detected, some of them were not. For those fragments that are detected, the sensitivity is generally good. The CITP format is ideal for MS/MS operation. We are confident that the CITP/MS combination will continue to develop in importance.

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