Nano-LC/MS of attomole digested Glutamate Dehydrogenase (GDG) with the polypropylene nanospray nozzle

For proteomic studies, nano-LC/MS is an indispensable tool. A stable spray of the LC eluent into the mass spectrometer over the entire range of mobile phase compositions is a pre-requisite for a successful experiment. At present, clogging and instability of the spray during the run are major factors leading to failed experiments and ruined samples. This application note describes an experiment using the polypropylene nanospray nozzle for the mass spectrometry interface. The spray stability and mass spectrometry sensitivity are evaluated.

Experimental

Column: 75 um inside diameter (i.d.), 360 μm outside diameter (o.d.) fused silica capillary was used. The frit was made according to the sol-gel Kasil protocol[1].
Commercial column with integral frit can also be used.
Column was packed with C8 (5 μm particles) to a depth of 7-8cm
5 cm column cleaved off (with an Upchurch silica column cutter (FX315)) for a clean, square cut end. This was especially important for the column end to be inserted leak-free into the nanospray nozzle
The column was conditioned by a run of 100 fmol of a digest of yeast enolase through the column.

Nanospray-Mass spectrometry interface:

1) Nanospray chip- If the chip was used, it was held in a holder that provided an electric ground to the front face of the chip. The end of the column was inserted into one of the 4 nozzles. Positioning of the chip was achieved through a 3-D positioning stage.
2) Nanospray nozzle- A stand-alone nozzle was cut from the chip. The column inserted into the back of the nozzle held the nozzle in position for optimized spraying. It was not necessary to have an electrical ground to the nozzle.
3) Electrical contact between the sample and the high spray voltage was made through a liquid T-junction before the column. A voltage of 1.3 KV was applied through a gold wire to the sample. The nozzle was placed 0.5 to 2 mm from the mass spectrometer inlet.
4) The mass spectrometer used was a ThermoFinnegan LTQ.

Mobile Phases for LC: the mobile phases:
A: H_20 + 0.5% Acetic Acid
B: Methanol + 0.5% Acetic Acid.

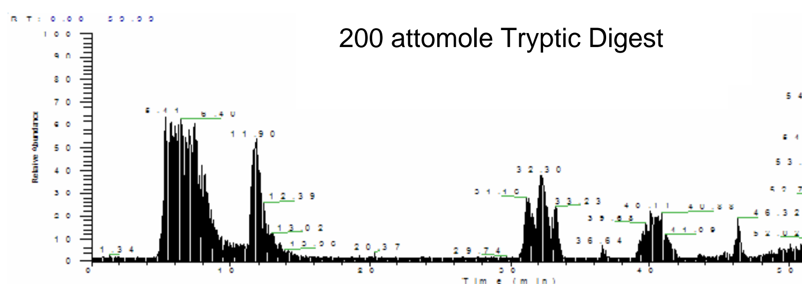
The run was as follows:
1)10%B for 15 minutes (sample loading and column washing),
2)10%-90%B gradient for 20 minutes
3) 90% B for 15 minutes
4) 2 minute gradient back to 10%B
5) Followed by a 10 minute re-equilibration

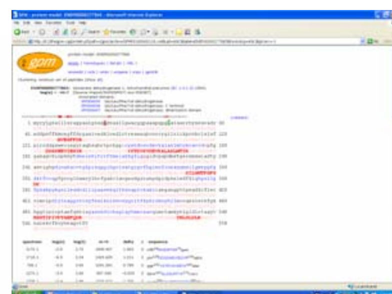
Data were collected over the entire 1-hour run, with the first approximately 40 minutes of the run containing useful data. Each set of chromatographs was followed by the result from the search engine (X!Tandem which can be accessed at www.thegpm.org). No data manipulation was performed on the spectra before the search.

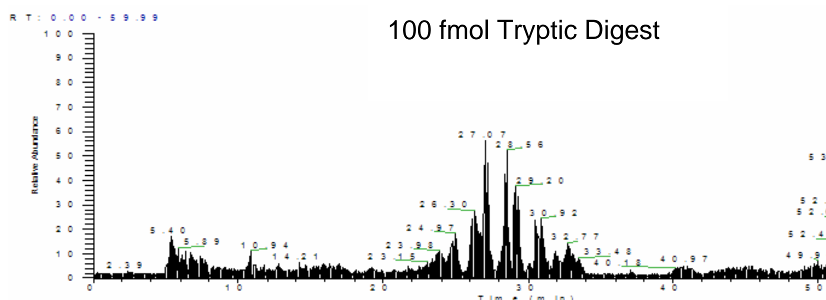
Single nozzle supported by column

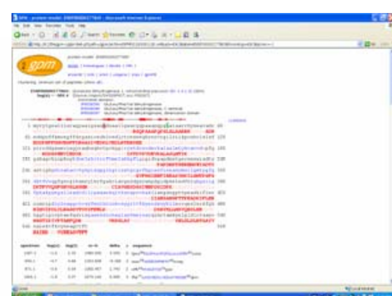
Nozzle placed close to inlet during run

Results of nano-LC/MS of digested Glutamate Dehydrogenase (GDG)









Discussion

The polypropylene nanospray chip was successfully used for the nanoLC-MS analysis of a digested enzyme at attomole quantities. Some peak broadening due to a ~29 nL dead volume behind the nozzle was apparent, but the spray was extremely stable over the entire mobile phase range, and the spray characteristics did not noticeably deteriorate with time or use. A single nozzle typically lasts for many months of regular use. The dead volume has been greatly reduced to ~ 0.5 nL by a column with a tapered end that fills up the extra space in the channel before the nozzle opening.

There has been great emphasis in LC-MS/MS on getting outstanding chromatography, but this is often done at the expense of electrospray. For example most practitioners use an acetonitrile based mobile phase for LC-MS/MS. However, almost nobody uses acetonitrile for offline "classic" nanospray. By using a methanol-based mobile phase (closer to the optimum solution for electrospray), we obtained superior nanospray even though the chromatography suffered somewhat in this experiment. Using C8 instead of C18 may further improve the quality of methanol-based chromatography

We thank Dr. Mike Myers, Cold Spring Harbor Laboratory, for providing the information in this application note.

Notes:

[1]The fused silica capillary was washed with MeOH and then dried. The sol-gel was made by mixing 170 ul of KASIL#1 (potassium silicate from the PQ Corporation) with 30 ul of formamide. The mixture was vortexed for 1 minute and then microfuged for one minute. One end of the capillary was quickly dipped into the mixture, which resulted in filling of the end by capillary action. The capillary was baked at 100 C to form the frit (3-4 hours up to overnight). Once the frit was formed, the end of the capillary was trimmed to leave a 2-3 mm long frit. The capillary was then washed with1 M HCl, then water, and finally 100% ACN (2 minutes in each solvent.) Optionally the frit was washed for 5 minutes with 1 M ammonium nitrate before the 1 M HCl wash to eliminate the potential of potassium leaching. Other protocols to put in frits based on sol-gel chemistries may also work. Watch for frit failure during the solvent washes.

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