Applied Biosystems

Use of ZipTip® Pipette Tips for Microscale Affinity Mass Spectrometry

Overview

Affinity chromatography forms the basis of the separation and purification of a number of biological molecules. For example, antibodies may be purified based on their affinity for protein A, or a protein may be purified on the basis of its affinity for a monospecific antibody raised against it. Typically, these purifications take place in a chromatographic column with a relatively large volume. This note discusses the use of the C_{18} ZipTip pipette tips for conducting affinity-based separations on a microliter scale.

Key Features

- Affinity Separation Protocol
- Examples of ZipTip Pipette Tip Affinity Separations

Experimental Conditions

- Accelerating Voltage: 25 kV
- Grid Voltage: 93%
- Mode: Linear
- Guide Wire Voltage: 0.1 to 0.3%
- Matrices: Sinapinic acid (SA) at 10 mg/mL in 50% MeCN/0.2% TFA, α-cyano-4-hydroxycinnamic acid at 10 mg/mL in 50% MeCN/0.2% TFA.

Affinity Separation Protocol

- Wash ZipTip pipette tip (MeCN, 50% MeCN, 0.1% TFA)
- Incubate ZipTip pipette tip with Affinity Agent, e.g. Ab
- Wash with incubation buffer
- Incubate ZipTip pipette tip with blocking agent, e.g. BSA
- Wash with incubation buffer
- Incubate with sample, e.g., antigen
- Wash with incubation buffer, then water
- Elute sample with acid, e.g., 1% TFA
- Elute with 50% MeCN

We followed the affinity separation protocol above. After conditioning the ZipTip pipette tip (Figure 1), a goat anti-human transferrin was incubated with it by passing 20 µL of anti-serum up and down the tip ten times. After 3 washes with 10 µL aliquots of phosphate buffered saline at pH 7.4 (PBS), 20 µL of a 20 mg/mL solution of bovine serum albumin (BSA) was passed up and down the tip ten times to block the remaining unbound C_{18} sites. Following another 3 PBS washes, 20 µL of filtered human breast milk or pure transferrin at 0.01 mg/mL was repeatedly passed into and out of the ZipTip pipette tip for approximately 5 minutes. Following this incubation 2 PBS washes were performed followed by a wash with double-distilled water. To elute the bound transferrin, 3 µL of 1% TFA was passed up and down the tip ten times and finally eluted onto the MALDI plate and mixed with matrix or eluted into a buffered solution of trypsin at 20 µg/mL in 25 mM ammonium bicarbonate. If the sample was eluted into trypsin, it was incubated overnight at 37ºC. An aliquot was mixed with CHCA matrix and deposited on the MALDI plate.

Figure 1. The ZipTip pipette tip.

Results and Discussion

After acid elution from the ZipTip pipette tip and MALDI-TOF mass spectrometry analysis, a peak at approximately 80 kDa was observed in the control experiment (pure transferrin) and the sample studied (breast milk) as shown in Figure 2, spectrum 1 and spectrum 3 respectively. Spectrum 2 represents the peaks observed after 50% MeCN elution from the ZipTip pipette tip, indicating that IgG was indeed bound to the tip. Spectrum 4 is human breast milk diluted 10-fold with sinapinic acid. Clearly, no transferrin was observed. Thus, specific binding of transferrin to the anti-transferrin ZipTip pipette tip was shown.

If the transferrin was eluted into trypsin and the resulting digest mass analyzed and data base searched, transferrin was clearly identified. Additional control experiments were performed to determine the specificity of the interaction between the transferrin and the anti-transferrin ZipTip pipette tip (Figure 3). These experiments were performed four times with similar results obtained each time.

To demonstrate that the interaction observed in Figure 2 was indeed specific and not just an interaction of the transferrin with the C_{18} resin or with the plastic tip, a simple control experiment was performed.

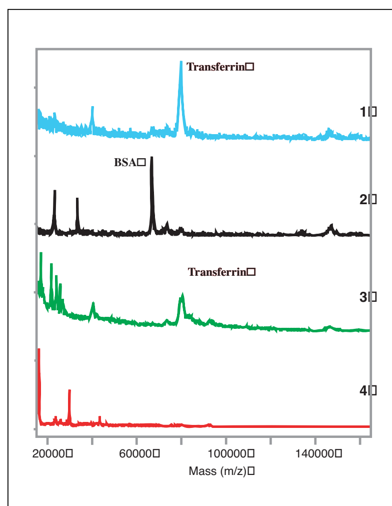

Figure 2. Zip Tip affinity purification of transferrin.

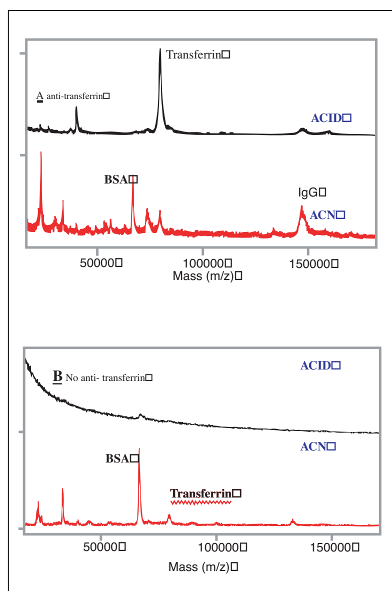

Figure 3. Demonstration of the specific nature of the interaction between the anti-transferrin ZipTip and transferrin.

The ZipTip pipette tip was incubated with or without anti-transferrin prior to blocking with BSA. In addition, 0.05% Tween 20 was either added or not added to the sample. Thus, one tip was an anti-transferrin tip and the other a BSA ZipTip pipette tip. In panel B of Figure 3, it can be seen in the bottom spectrum (organic elution) that little transferrin was bound in the absence of anti-transferrin IgG. However, when anti-transferrin was present, transferrin was strongly detected after acid elution (panel A, acid elution). In the absence of Tween 20, stronger non-specific binding was observed (data not shown).

Conclusion

When coupled to MALDI-TOF mass spectrometry, the ZipTip pipette tip makes an excellent platform for microscale affinity purification and identification.

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