Affinity Capture and MALDI-MS of Cytokines from Mouse Serum

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Inflammation is a condition associated with a wide variety of diseases.[1] The inflammatory response involves changes in serum levels of some cytokines. We are working toward a method for screening cytokine levels in mutant mouse populations as a possible means for earlier detection of genotypes leading to chronic inflammatory diseases. We describe a method for purifying targeted cytokines from serum and cell culture supernatants using affinity capture followed by MALDI-MS analysis.[2]

Experimental: Antibodies to various cytokines were attached to 1 μm diameter aminopolystyrene beads.[3] The beads were incubated with cell culture supernatants, or a solution of a recombinant version of the cytokine(s) of interest in 5 mg/mL BSA in PBS. The beads were recovered by centrifugation and washed. Captured cytokines were eluted from the beads by adding MALDI matrix solution, either sinapinic acid (SA) or α-cyano-4-hydroxycinnamic acid (CHCA) in 50% acetonitrile/50% aqueous 0.1% trifluoroacetic acid. The supernatant was transferred to a MALDI sample plate pre-treated with a thin layer of the appropriate matrix deposited from a volatile solvent.[4,5] MALDI-TOF measurements were performed using either a Voyager DE or Voyager Elite (PerSeptive Biosystems). Multiple spectra were obtained from each sample spot and those with highest S/N were subsequently averaged.

Results: Figure 1 shows MALDI spectra of several recombinant cytokines (R&D Systems), each at the 100 ng level. The spectra were obtained using SA matrix, except interleukin-6 (IL-6), for which better sensitivity was obtained using CHCA. Multiple peaks in the interleukin-2 (IL-2) spectrum are consistent with the presence of both N-terminal methionyl and native versions of the protein in the commercial product. The reason is not clear for large difference between the measured and calculated m/z for interferon-γ (IFN–γ). A multiplex-capture experiment was performed (data not shown) in which a mixture of affinity beads, each derivatized with an antibody to tumor necrosis factor-α (TNF–α), IL-2, or IFN-γ, was challenged with a mixture of these three cytokines. All three cytokines were detected at the 20 ng level. However, spectral overlap was observed between TNF-α and the largest IL-2 species, which differ in mass by only 17 Da. Multiplexing is desirable because cytokines can act in concert in biological systems.[1]

Figure 2 shows results from affinity capture of native TNF-α from supernatants of a series of lipopolysaccharide (LPS)-treated mouse cell (macrophage or spleen) cultures. ELISA measurements of TNF-α were performed before and after capture, as shown in the figure. The qualitative trend is that a larger TNF-α signal is observed in the MALDI spectrum from those samples containing higher amounts of TNF-α by ELISA. Some non-specific capture was observed, but it did not interfere with detection of the TNF-α; we observed similar results from spiking recombinant TNF-α into mouse serum, followed by affinity capture and MALDI analysis.[3] Higher-mass forms (including glycoforms)[6] of TNF-α were not observed in this

experiment. Spectra in Figure 2 were obtained using the solution phase of the MALDI matrix-eluted capture beads; very similar results were obtained by transferring the remaining capture beads themselves (with matrix solution) to the MALDI plate. The relative intensities of species observed in the spectra were retained, and the observed spectral resolution was unaffected by the presence of the 1-μm diameter capture beads on the MALDI plate.

The sensitivity currently achievable by MALDI-TOF for captured TNF-α is approximately 10 ng/mL, which is considerably poorer than ELISA sensitivity. However, the direct m/z measurement obtained with mass spectrometry offers considerable advantages, allowing detection of, for instance, a mutant form or post-translational modification[7,8] of the cytokine. Experiments using radiolabeled TNF-α showed that the affinity capture itself is useful to <100 pg/mL.[3] We are currently working toward improved sensitivity for the MALDI analysis, by (1) using nanoliter deposition techniques to confine the dried spot to a smaller area, and (2) performing post-capture enzymatic digests to yield peptides that provide a more intense MALDI response than the parent proteins.

Acknowledgements: Research sponsored by the Laboratory Directed Research and Development Program of Oak Ridge National Laboratory (ORNL), and the Office of Biological and Environmental Research, U.S. Department of Energy. ORNL is managed and operated by UT-Battelle, LLC, for the U.S. Department of Energy under contract DE-AC05-00OR22725.

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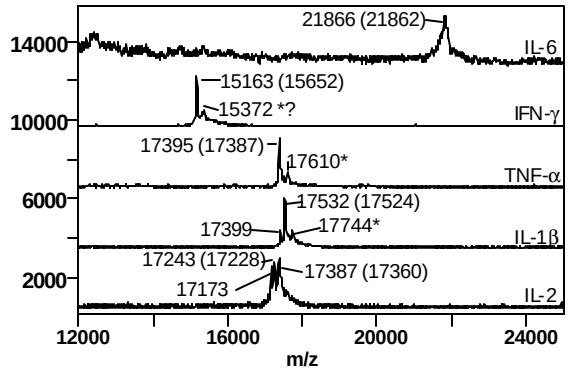

Figure 1. MALDI spectra of recombinant cytokines. Calculated m/z values are shown in parentheses beside experimental values. Sinapinic acid adducts are indicated with *.

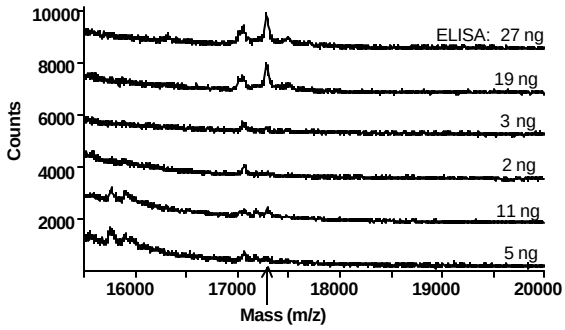

Figure 2. Capture of native TNF-α from LPS-stimulated cell culture supernatants. Approximate amounts captured were estimated by ELISA. Arrow on x-axis shows calculated TNF-α m/z.