

Determination of superoxide dismutase (SOD) by using species-specific isotope dilution (SS-IDMS) analysis using GE-LA-ICP-MS

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The quantification of proteins can as best described as semi-quantitative methods and bioanalytical chemistry has so far been satisfied. However, a direct comparison between two different sets of samples (e.g., exposed and control) are necessary. Absolute measurements can not be made. Using elemental mass spectrometry is capable to determine in absolute terms the amount of a metal-containing species when separated from other species. During separation and preparation steps the integrity of the compound cannot always been guaranteed. Hence, new techniques need to be introduced. The quantification of organometallic compounds in biological samples has made a step change when the concept of species-specific isotope dilution mass spectrometry (SS-IDMS) has been used for example for butyltin or methylmercury compounds.

Here this concept is used for the quantification of the metalloprotein superoxide dismutase (SOD), which contains one copper and one zinc in each of the two subunits. The absolute quantification of SOD is necessary to assess the redox stress from so called reactive oxygen species (ROS) in a certain tissue. A copper and zinc isotopically-enriched superoxide dismutase (SOD) which has been used to quantify SOD in liver homogenates using 2 D chromatography ICPMS has been used as spike.^{1,2} Here we applied SS-IDMS for the separation of protein mixtures on a non-denaturing gel electrophoresis coupled with laser ablation inductively-coupled plasma mass spectrometry (GE-LA-ICPMS) as described elsewhere³.

The isotopically enriched SOD shows the same migration time as the native SOD in the 1 D gels. When measuring the isotope ratio of copper and zinc using LA-ICPMS from the gel it is obvious that the proteins do not only migrate horizontal to the migration paths but they show also diffusion into the gel in orthogonal direction.

Considering the orthogonal diffusion of the proteins recovery rates around 89 % can be achieved for SOD when SS-IDMS-GE-LA-ICPMS is used.

¹ C.L. Deitrich, A. Raab, B. Pioselli, J. Thomas-Oates, J. Feldmann, Chemical preparation of an isotopically enriched superoxide dismutase and its characterisation as a standard for species-specific isotope dilution analysis, **Analytical Chemistry** (2007) **79**, 8381-8390.

2. B. Pioselli, C. Munro, A. Raab, K. Songsrurute, J. Feldmann, J. Thomas-Oates, Denaturing and non-denaturing microsolution isoelectric focussing to mine the metalloproteome, **Metallomics**. In press August (2009)

3. A. Raab, B. Pioselli, C. Munro, J. Thomas-Oates, J. Feldmann, Evaluation of gel electrophoresis conditions for the separation of metal tagged proteins with subsequent laser ablation ICP-MS detection, **Electrophoresis** (2009), **30**, 303-314

